

## Review Article

# Genetics of Proteasome Diseases

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The proteasome is a large, multiple subunit complex that is capable of degrading most intracellular proteins. Polymorphisms in proteasome subunits are associated with cardiovascular diseases, diabetes, neurological diseases, and cancer. One polymorphism in the proteasome gene *PSMA6* (−8C/G) is associated with three different diseases: type 2 diabetes, myocardial infarction, and coronary artery disease. One type of proteasome, the immunoproteasome, which contains inducible catalytic subunits, is adapted to generate peptides for antigen presentation. It has recently been shown that mutations and polymorphisms in the immunoproteasome catalytic subunit *PSMB8* are associated with several inflammatory and autoinflammatory diseases including Nakajo-Nishimura syndrome, CANDLE syndrome, and intestinal *M. tuberculosis* infection. This comprehensive review describes the disease-related polymorphisms in proteasome genes associated with human diseases and the physiological modulation of proteasome function by these polymorphisms. Given the large number of subunits and the central importance of the proteasome in human physiology as well as the fast pace of detection of proteasome polymorphisms associated with human diseases, it is likely that other polymorphisms in proteasome genes associated with diseases will be detected in the near future. While disease-associated polymorphisms are now readily discovered, the challenge will be to use this genetic information for clinical benefit.

## 1. Introduction

Over the last decade, significant improvements have been made in genotyping efficiency, sequencing technology, and statistical methodology, providing researchers with better opportunities to define the role of sequence variation in the development of human diseases [1–3]. Many human diseases are now known to have a genetic component. All humans start their lives with germ-line mutations inherited from their parents. However, the human genetic code is constantly subjected to mutations which can happen during cell division or after exposure to environmental factors such as UV radiation, chemicals, or viruses. These mutations can result in proteins with altered functions, malformed proteins, or even missing proteins. Some of these changes that occur due to a particular mutation have no effect on biological function (silent mutations), some may be beneficial, and some may lead to disease. These genetic variations are important for genetic diversity within the population.

Genome-wide association (GWA) studies have identified alleles related to complex disorders; however some of

these alleles seem to be associated with the disease only in certain populations. Most investigations use dense maps of single-nucleotide polymorphisms (SNPs) as well as the haplotypes derived from these polymorphisms. Determining the underlying causal relationship between SNP or haplotype and disease is currently a major challenge. Polymorphisms (termed “alleles”) occur more often (frequency of 1% or greater) in the general population than mutations [4, 5]. Single-nucleotide polymorphisms (SNPs) are the most common type of polymorphism and account for 90% of human DNA polymorphisms. Most SNPs have two alleles which are designated “major” and “minor” based on their observed frequency in the general population. At each SNP, several genotypes are possible because chromosomes are both maternal and paternal in origin: homozygous for the major allele, heterozygous, or homozygous for the minor allele. It is estimated that more than 10 million SNPs occur in our whole genome (once every 100–300 bases) [6]. Because of the large number of SNPs in the whole genome, investigation of all the SNPs for a large number of individuals is time-consuming and costly. Whole genome sequencing for large sample

numbers is also not desirable because many SNPs are rare and occur only once ("singletons") or twice ("doubletons") in the analyzed samples.

The haplotype refers to an individual collection of short tandem repeat allele mutations at adjacent locations (loci) that are inherited together. Genome scan approaches to find regions associated with diseases are now much more efficient due to efforts such as the HapMap [6]. The HapMap contains maps of haplotype blocks and their SNPs, allowing users to select a group of SNPs to investigate a possible association between known genomic regions and the disease being studied. Smaller research labs are now able to analyze multiple genes belonging to the same pathway instead of analyzing a single polymorphism on a single gene. These advances have led to the discovery of new polymorphisms on proteasome genes that are linked to major human diseases.

**1.1. The Ubiquitin-Proteasome System (UPS).** The UPS is the major pathway for degrading intracellular proteins. The number of cellular processes that the UPS system is involved in is impressive and includes cell cycle regulation, cellular differentiation, removal of abnormal and misfolded intracellular proteins, and generation of antigenic peptides [7–10]. The first step in UPS-mediated protein degradation involves ubiquitination, which acts as a signal for degradation and is carried out by a series of enzyme-mediated reactions involving at least three types of enzymes, E1, E2, and E3 (Figure 1). The ubiquitin-activating enzyme (E1) generates activated ubiquitin (Ub) via an ATP-dependent mechanism. Activated Ub is transferred to the ubiquitin-conjugating enzymes (E2), which, together with ubiquitin protein ligases (E3), ligates Ub to lysine residues on protein substrates [11]. This process of ubiquitination occurs multiple times resulting in ubiquitinated substrates which are recognized by the proteasome or proteasome associated proteins. Once bound to the proteasome, the polyubiquitin tag on the substrate is removed by deubiquitinases which allows the Ub to be recycled in the cell. The deubiquitinated substrate is then unfolded and translocated into the 20S core by the 19S regulatory particle. Once inside the core, the substrate is degraded by the proteolytic enzymes of the 20S proteasome. The proteasome contains three proteolytic activities: caspase-like ( $\beta$ 1i), trypsin-like ( $\beta$ 2i), and chymotrypsin-like ( $\beta$ 5i) activity.

The importance of the proteasome in cellular functions is exemplified by experimental evidence which suggests that the proteolytic capacity of the proteasome in certain tissues declines with age and that this decline in proteasome activity is related to the lifespan of the organism [12–16]. In contrast, long-lived naked mole rats and centenarians show elevated proteasome levels and activity [15, 16]. Aging cells have increased levels of damaged proteins, possibly increasing the load on the proteasome [17]. This proposed imbalance between proteasome activity and proteasome substrate load has been suggested to be responsible for the buildup of protein aggregates in aged cells. The impact of proteasome proteolytic capacity on the replicative lifespan in *Saccharomyces cerevisiae* was investigated using a genetic system that

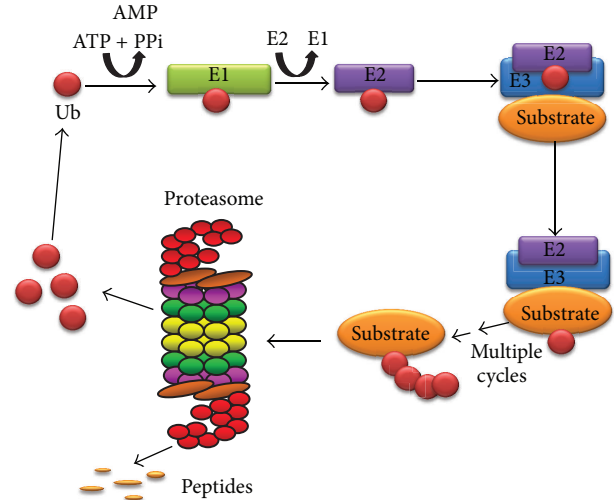


FIGURE 1: Schematic diagram of the ubiquitin-proteasome system. The UPS involves at least three enzymes (E1, E2, and E3) that catalyze the addition of ubiquitin to lysine residues on the substrate protein. Polyubiquitinated substrates are then recognized by the proteasome or proteasome associating protein, and the ubiquitin removed by deubiquitinases and the substrate unfolded and translocated in the 20S core for proteolysis.

allowed the abundance of UPS components to be manipulated at the transcriptional level [18]. Increasing the levels of the UPS-related transcription factor Rpn4 upregulates UPS components and enhances replicative lifespan and resistance to proteotoxic stress. This effect of increased proteasome capacity on lifespan is independent of the proteotoxic stress response [18].

In a yeast model for neurodegenerative diseases, elevated proteasome capacity results in improved clearance of toxic Huntington fragments, suggesting that lifespan extension may be related to elimination of damaged proteins in old cells [18]. Overexpression of the proteasomal deubiquitinating subunit Rpn11 extends lifespan in flies [19]. In *C. elegans* the downregulation of proteasome regulatory particle subunits leads to a substantial shortening of lifespan [20].

**1.2. Proteasome Components.** The proteasome is a multicatalytic enzyme which is highly conserved. The predominant intracellular form is composed of two large complexes, the 20S and 19S complexes (Figure 2) [21–23]. Proteasomes are found in archaeobacteria as well as the nucleus and cytoplasm of all eukaryotic organisms. The proteasome complex is essential for cellular processes, as removal of any proteasome gene is lethal in eukaryotes [24, 25]. The 20S proteasome, or core particle, contains the proteolytic sites responsible for protein degradation. The 20S proteasome is a 28-subunit barrel-like structure of four rings of subunits (two  $\alpha$  and two  $\beta$  rings, arranged  $\alpha\beta\beta\alpha$ ), with each ring containing seven subunits. Each  $\alpha$  and  $\beta$  subunit occurs in duplicate and three of the  $\beta$  subunits have proteolytic capabilities:  $\beta$ 1 (encoded by *PSMB6* gene), with caspase-like proteolytic activity;  $\beta$ 2 (*PSMB7*), with trypsin-like activity; and  $\beta$ 5 (*PSMB5*), with

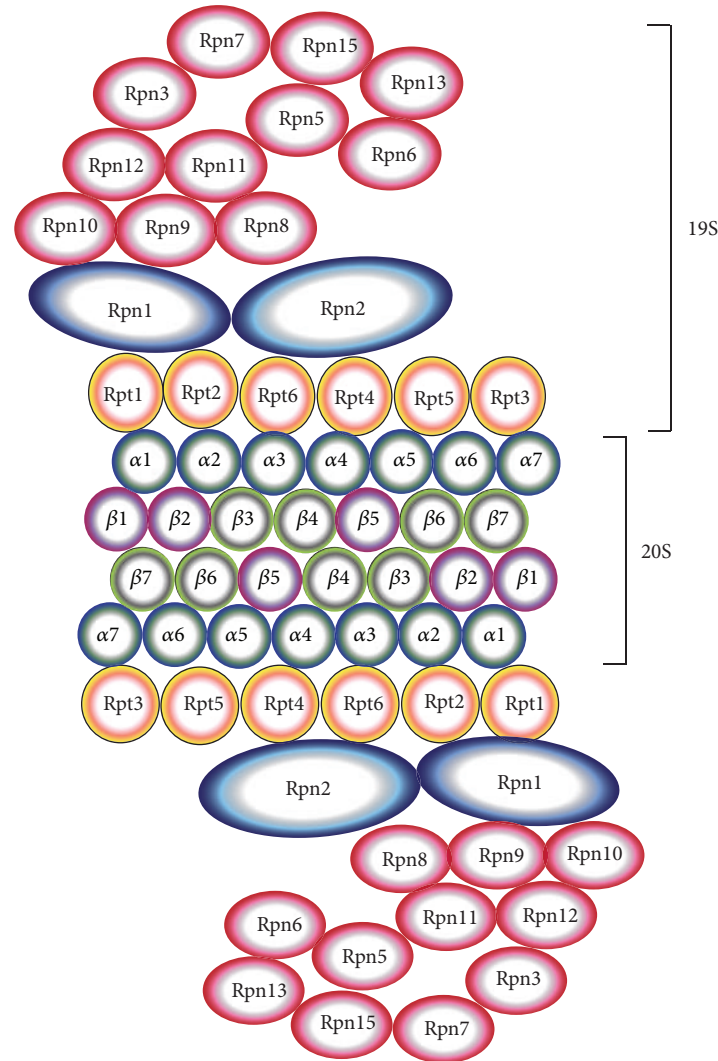


FIGURE 2: Schematic diagram of the 26S proteasome. The 26S proteasome is composed of the core 20S proteasome and the 19S proteasome complex.

chymotrypsin-like activity. The gene and protein names of the components of the proteasome are shown in Table 1. The 19S proteasome complex, or regulatory complex, is important in mediating substrate recognition, processing, and transporting substrates into the catalytic chamber of the 20S core [26]. Modulation of the 20S and 26S proteasomes by posttranslational modifications has been shown to affect proteasome activity [27]. The 19S is structurally more complex than the 20S, with six different ATPases that unfold globular proteins, non-ATPase regulatory subunits that bind polyubiquitin chains, and non-ATPase regulatory subunits that cleave ubiquitin moieties off of polyubiquitinated proteins. The ATP-dependent 19S regulatory complexes are involved in unfolding and translocating polyubiquitinated substrates into the interior of the 20S complex. Once inside the 20S core substrates are degraded into oligopeptides. While ATP hydrolysis is not needed to cleave the substrate peptide bonds, ATP is needed for substrate unfolding and translocation into the proteasome's 20S core chamber. The 19S proteasome can be

replaced by other proteasome activator complexes (also called 11S, encoded by *PSME* genes), or PA200 (*PSME4*) [28–30]. PI31 (*PSMF1*) inhibits the activation of the 20S proteasome by 19S and 11S and inhibits hydrolysis of protein and peptide substrates by the 20S proteasome [31, 32]. Intracellularly, multiple forms of the proteasome with different combinations of activators coexist (Figure 3). These different forms have different proteolytic activities and functions and are likely to be an important contributing factor in diseases.

Two other 20S proteasome genes, *PSMA8* and *PSMB11* (codes for  $\beta 5t$ ), which occur in specific tissues, were recently reported but are not currently known to be associated with any diseases [33, 34]. In mammalian testis, most proteasomes contain a spermatid/sperm-specific  $\alpha$  subunit *PSMA8* and the PA200 activator [33]. These mammalian testis proteasomes, called spermatoproteasomes, are important for the polyubiquitin-independent degradation of histones. Another catalytic proteasome subunit,  $\beta 5t$ , was found to be expressed exclusively in cortical thymic epithelial cells

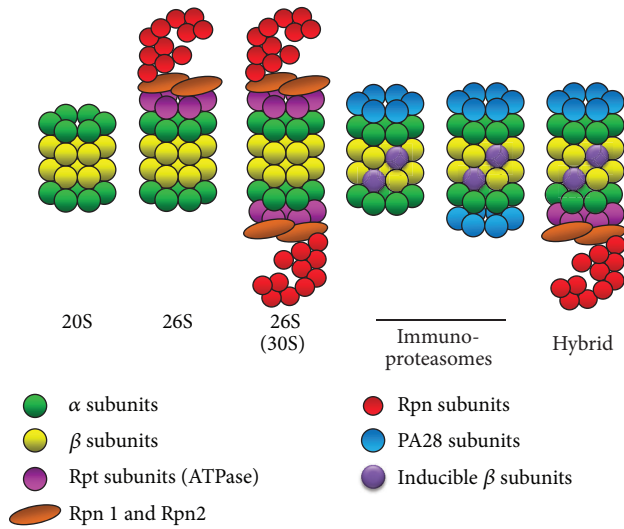


FIGURE 3: Schematic diagram of different forms of the proteasome. Intracellular proteasome exists in different forms. The 26S proteasome can exist with one or two 19S caps, immunoproteasomes containing one or two 11S caps, proteasomes containing the 20S proteasome with one or two PA200 caps (in the nucleus only), and hybrid proteasomes which contain different combinations of 20S and activators.

[34]. The replacement of  $\beta 5$  or  $\beta 5i$  with  $\beta 5t$  selectively reduces chymotrypsin-like activity of the proteasome [34]. The thymoproteasome (proteasome with  $\beta 5t$ ) is important for development of CD8(+) T cells in the thymus, as it plays a key role in generating the MHC class I-restricted CD8(+) T cell repertoire during thymic selection [34, 35].

## 2. 20S Proteasome Mutations and Polymorphisms

While all *PSMA* and *PSMB* genes have known gene mutations [38], only a few 20S proteasome genes have detected polymorphisms that are associated with disease. Table 2 shows the polymorphisms in proteasome genes that are associated with diseases. Alignment of human *PSMA* (Figure 4) and human *PSMB* (Figure 5) protein sequences shows that *PSMA* and *PSMB* proteins have some homology with each other. Phylogenetic analyses of *PSMA* and *PSMB* protein subunits show that all subunits are evolutionarily related to each other (Figure 6). Current evidence suggests that two constitutive 20S genes, *PSMA6* and *PSMA7*, have polymorphisms associated with human diseases.

**2.1. *PSMA6*.** The proteasome gene, *PSMA6*, codes for a 246 residue protein called  $\alpha 1$ . This protein is structurally important in forming the outer  $\alpha$  rings of the 20S core proteasome. The  $\alpha 1$  protein function is also likely to be modulated by posttranslational modifications including phosphorylation, glycosylation, and lysine acetylation [60, 84]. In humans, *PSMA6* is most closely related to *PSMA4* and *PSMA2* (Figure 6). The location of the *PSMA6* gene occurs in a region containing microsatellites that have been implicated in

coronary artery disease (CAD) [85], type 2 diabetes mellitus (T2DM) [86], and Grave's disease [87].

**2.1.1. Coronary Artery Disease.** No association between two SNPs (rs1048990 and rs12878371) in the *PSMA6* gene, as well as two SNPs in the *KIAA0391* gene and one SNP downstream of both genes, with CAD in a Saudi population (1071 patients and 929 controls) was detected [85]. These two genes, *KIAA0391* and *PSMA6*, which have both been reported to predispose individuals to CAD, form an evolutionarily conserved cluster in the chromosomal region 14q13.2. Interestingly, two haplotypes in the chromosomal region (five SNPs in a 100 kb region of chromosome 14) encompassing *KIAA0391* and *PSMA6* genes, 1A-2G-3C-4A-5A and 1A-2G-3G-4A-5A, show increased risk of both CAD and myocardial infarction (MI), while another haplotype, 1T-2G-3C-4G-5A, showed decreased risk of CAD and MI [85]. These latter results suggest that disease risk factor determination may be improved by investigating haplotypes instead of SNPs. Other recent experimental data suggest that haplotypes are more predictive than individual SNPs at determining risk factors for complex diseases [90].

CAD is a complex disease, and several molecular pathways as well as loci and candidate genes that affect the susceptibility to CAD have been suggested to be involved. A functional sequence variation,  $-8C/G$ , in *PSMA6* was found to increase susceptibility to CAD [72]. 713 Caucasian ischemic stroke patients (708 controls) and 166 African American ischemic stroke patients (117 controls) were investigated using odds ratios (ORs) from multivariable logistic regression models for twenty SNPs previously shown to be associated with MI or CAD [72]. The *PSMA6*  $-8C > G$  (SNP rs1048990) was found to have a protective association with ischemic stroke in both Caucasians and African Americans (i.e., decreased risk of ischemic stroke). Investigation of 1330 cases and 2554 controls from Japanese and Korean populations for *PSMA6* genotypes showed no evidence of the association with either population [43]. An investigation of 6946 MI patients and 2720 unrelated controls showed that the homozygous GG genotype for the  $-8C > G$  polymorphism was less frequent in the UK population (2.1%) than in the Japanese population (8.9%) [91]. No association between the *PSMA6* polymorphism and MI was found in the British population. Another genetic association study on *PSMA6*  $-8C/G$  using 210 North Indian CAD patients and 232 controls did not show any association between the *PSMA6* variant and CAD [92].

**2.1.2. Myocardial Infarction.** In a case-control association study of 1884 MI Chinese patients and 2643 unrelated controls, genotyping of the *PSMA6*  $-8C > G$  polymorphism showed that this SNP was associated with MI [73]. No relationship between *PSMA6*  $-8C > G$  and sex, age, or other conventional cardiovascular risk factors was detected. A recent meta-analysis of 15,991 cases and 16,784 controls from ten case-control studies suggest that the  $-8C/G$  sequence variation is a risk factor for increased CAD susceptibility, but the association between the sequence variation and CAD



TABLE 1: Names and characteristics of human proteasome genes.

Gene name	Protein name	Other names	Chromosome location	Sequence length	MW (Da)	First methionine removed	Polymorphisms	Reference
20S subunits								
PSMA1	$\alpha 6$	C2, Pro- $\alpha 5$ , $\alpha 6$ .sc, nu, Pros30, p30k, Pre5, HC2, PSC2	11p15.1	263	29556	No	rs17850016 (G37V)	[36, 37]
PSMA2	$\alpha 2$	C3, Pro- $\alpha 2$ , $\alpha 2$ .sc, Pre8, Prs4, Y7, HC3, PSC3, Lmpc3	7p14.1	233	25767	Yes	(L110V)	[38, 39]
PSMA3	$\alpha 7$	C8, Pro- $\alpha 7$ , $\alpha 7$ .sc, Pre10, Prs1, Cl, Prcl, HC8, PSC8	14q23	254	28302	Yes		[40]
PSMA4	$\alpha 3$	C9, Pro- $\alpha 4$ , $\alpha 3$ .sc, Pre9, Prs5, Y13, HC9, PSC9	15q25.1	261	29484	No		[41]
PSMA5	$\alpha 5$	Zeta, Pro- $\alpha 1$ , $\alpha 5$ .sc, Pip2, Doa5, [Pup2]	1p13	241	26411	No		[42]
PSMA6	$\alpha 1$	Iota, Pro- $\alpha 6$ , $\alpha 1$ .sc, Pros27, pre27k, C7, Prs2, Y8, Scl1	14q13	246	27399	No	rs1048990 (-8C-G), rs15434 (A233S)	[37, 43, 44]
PSMA7	$\alpha 4$	C6, Pro- $\alpha 3$ , $\alpha 4$ .sc, XAPC-7, Pre6	20q13.33	248	27887	No	335C-A (A112D)	[38, 45]
PSMA8	—	PSMA7L	18q11.2	256	28530	No		[46]
PSMB1	$\beta 6$	C5, gamma, Psc5, Pre7, Prs3, Pts1	6q27	241	26489	No	rs12717 (P11A), rs10541 (I208N)	[36, 47]
PSMB2	$\beta 4$	C7, Pre1, C11, C7-I, HC7-I	1p34.2	201	22836	No		[48]
PSMB3	$\beta 3$	C10, theta, Pup3, C10-II	17q12	204	22818	Yes	rs4907 (M34L)	[48, 49]
PSMB4	$\beta 7$	N3, beta, Pros26, HsN3, Pre4, Rn3, Lmp3	1q21	264/219*	29204/24392*	No	rs1804241 (M95I), rs4603 (I234T)	[46, 48–51]
PSMB5	$\beta 5$	X, epsilon, LmpX, MB1, Pre2, Doa3, Prg1	14q11.2	263/204*	28480/22458*	No	rs11543947 (R24C)	[44, 48, 52]
PSMB6	$\beta 1$	Y, delta, LmpY, Pre3, Lmp19	17p13	239/205*	25358/21904*	Yes	rs2304974 (P107A)	[44, 52]
PSMB7	$\beta 2$	Z, alpha, Pup1, Mmc14	9q34.11-q34.12	277/234*	29965/25295*	No	rs4574 (V39A)	[36, 53]
PSMB8	$\beta 5i$	Lmp7, Psmb5i, Ring10, Y2, Cl3, Mcl3	6p21.3	276/204*	30354/22660*	No	rs114772012 (G8R), (PGH30-32RPD), rs2071543 (Q49K), rs17220206 (T74S), (T75M), (G201V)	[46, 54–57]
PSMB9	$\beta 1i$	Lmp2, Psmb6i, Ring12	6p21.3	219/199*	23264/21276*	No	rs35100697 (G9E), rs241419 (V32I), rs17587 (R60H), rs17213861 (R173C)	[58, 59]
PSMB10	$\beta 2i$	MECL-1, Lmp10	16q22.1	273/234*	28936/24648*	No		[60]
PSMB11	$\beta 5t$	beta5i-like, beta5t	14q11.2	300/251*	32530/27232*	No	rs34457782 (G49S)	[34]

TABLE 1: Continued.

Gene name	Protein name	Other names	Chromosome location	Sequence length	MW (Da)	First methionine removed	Polymorphisms	Reference
19S proteasome								
PSMC1	Rpt2	S4, Yhs4, Yta5, P26s4	14q32.11	440	49185	Yes		[38]
PSMC2	Rpt1	S7, Mss1, Yta3, Cim5, Nbla10058	7q22.1-q22.3	432	48503	Yes		[61]
PSMC3	Rpt5	S6a, S6', p50, Tbp1, Yta1, Sata	11p11.2	439	49204	No		[61]
PSMC4	Rpt3	S6b, S6, Mip224, Tbp7, Yta2, Ynt1, Cip21	19q13.11-q13.13	418	47336	No		[61]
PSMC5	Rpt6	S8, p45, Trip1, Sug1, Cim3, Crl3, TbpY, Tby1	17q23.3	405	45495	Yes	(R60Q), rs11543211 (R258W)	[39, 44]
PSMC6	Rpt4	S10b, p42, Sug2, Prs10, Pcs1, Crl13, CADP44, P44	14q22.1	389	44173	No		[38]
PSMD1	Rpn2	S1, p112, Sen3	2q37.1	953	105836	No		[60]
PSMD2	Rpn1	S2, p97, Trap2, Hrd2, Nas1, Rpd1, Protein 55.11	3q27.1	908	100200	No	rs11545172 (A176T), rs11545169 (E313D), rs17856236 (N724Y)	[44, 61]
PSMD3	Rpn3	S3, p58, Sun2, P91a, Tstap91a	17q21.1	534	60978	No		[60]
PSMD4	Rpn10	S5a, ASF1, Mcl1, Sun1	1q21.3	377	40737	No		[61]
PSMD5	—	S5b, KIAA0072	9q34.11	503	56065	Yes	rs2297575 (E21G), rs17282618 (L72H)	[60, 61]
PSMD6	Rpn7	S10a, SGA-113M, p44S10, p42A, PFAAP4, KIAA0107	3p21.1	389	45531	No		[60]
PSMD7	Rpn8	S12, p40, Mov34L	16q23-q24	324	37025	No		[62]
PSMD8	Rpn12	S14, p31, Nin1	19q13.2	350	39612	No		[60]
PSMD9	—	S15, p27	12q24.31-q24.32	223	24682	No	rs2230681 (V17A), rs2291116 (T74I), rs1177573 (R134W), rs1177573 (E197G)	[50, 63–65]
PSMD10	Gank-yrin	p28, p28(GANK)	Xq22.3	226	24428	No		[38]
PSMD11	Rpn6	S9, p44.5, Nas4	17q11.2	421	47333	Yes		[38]
PSMD12	Rpn5	p55, Nas5	17q24.3	455	52773	Yes	rs2230680 (V358A)	[60]
PSMD13	Rpn9	S11, p40.5, Les1, Nas7	11p15.5	376	42945	No	rs1045288 (N13S), rs28927679 (S150L), rs1794108 (G204E), rs1794109 (L205F)	[36, 66–68]

TABLE 1: Continued.

Gene name	Protein name	Other names	Chromosome location	Sequence length	MW (Da)	First methionine removed	Polymorphisms	Reference
PSMD14	Rpn11	Poh1, Mpr1, Mad1, Pad1, PAD1 homolog 1,	2q24.2	310	34577	No		[38]
Proteasome activators								
PSME1	PA28 $\alpha$	PA28A, IFI5111, 1S REG-alpha	14q11.2	249	28723	No	rs1803830 (S55N), rs14930 (T244K)	[48, 52]
PSME2	PA28 $\beta$	PA28B, 1S REG-beta	14q12	238	27270	Yes	rs7146672 (H89P)	[48, 60, 69]
PSME3	PA28 $\gamma$	PA28G	17q21.31	253	29375	Yes		[60, 70]
PSME4	PA200	KIAA0077, 1S REG-gamma	2p16.2	1843	211334	No	rs2302878 (I872V), rs805408 (S1371T), rs35903236 (T1825A)	[38, 44]
Proteasome inhibitor								
PSMF1	PI31		20p13	271	29817	No	rs1803415 (F36C), rs2235587 (H176R)	[60, 71]

\* Mature form of protein after propeptide is removed. When the first residue (methionine) of some proteins is removed, the molecular weights and sequence length given represent the mature forms of the proteasome subunit with the methionine removed.

TABLE 2: Polymorphisms in proteasome genes associated with human diseases.

Gene	Polymorphism	Amino acid change	Disease	References
20S subunits				
PSMA6	-8C>G (rs1048990)	—	Myocardial infarction	[60, 72, 73]
			Type 2 diabetes	[74, 75]
			Ischemic stroke	[72]
			Coronary artery disease	[60]
PSMA7	335C>A	A112D	Intellectual disability	[45]
19S subunits				
PSMD3	SNPs rs4065321 and rs709592	—	Diabetes	[76]
PSMD7	SNP, rs17336700 in intron 3	—	Ankylosing spondylitis	[18]
Immunoproteasome subunits				
PSMB8	c.224C>T, c.405C>A	T75M	JMP syndrome	[54]
		G210V	Nakajo-Nishimura syndrome	[57]
		T75M	CANDLE syndrome	[77]
		Q145K	<i>M. tuberculosis</i> infection	[78]
		—	Cancer	[79]
		—	Ankylosing spondylitis	[80]
PSMB9	G/T-37360	—	Type 1 diabetes mellitus	[81]
		—	Graves' disease	[82]
	179G>A	R60H	Ankylosing spondylitis	[83]

Table shows only disease-associated polymorphisms for which the SNP or amino acid change is known.

1	<u>-MFLTRSEYDRGVNTFSPEGRLFQVEYAIEAIKLG-STAI</u>	58	P28066	PSMA5
1	<u>-----MSYDRAITVFS</u> <u>PDGHLFQVEYAQ</u> <u>EAVKKG-STAV</u>	53	O14818	PSMA7
1	<u>---MASRYDRAITVFS</u> <u>PDGHLFQVEYAQ</u> <u>EAVKKG-STAV</u>	55	Q8TAA3	PSMA8
1	<u>MSRGSSAGFDRHITIFS</u> <u>PEGRLYQVEYAFKAINQ</u> <u>GGLTSVAVRGKDCAVIVTQKKVPDKL</u>	60	P60900	PSMA6
1	<u>---MAERGYSFSLTTF</u> <u>SPGKLVQIEYALAAVAGG-APSVGI</u>	56	P25787	PSMA2
1	<u>---MSRRYDSRTTIFS</u> <u>PEGRLYQVEYAMEAIGHA-GTCL</u>	55	P25789	PSMA4
1	<u>-MSSIGTGYDLSASTF</u> <u>SPDGRVFQVEYAMKA</u> <u>AVENS-STAI</u>	58	P25788	PSMA3
1	<u>---MFRNQYDNDVT</u> <u>VWSPQGRIHQIEYAMEAVKQG-SATV</u>	56	P25786	PSMA1
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59	MEPSSIE-KIVEIDAHIG-----CAMSLIADAKTLIDKARVETQNHWF	111	P28066	PSMA5
54	<u>QDERTVR-KICALDDNV</u> <u>CMAFA-----GLTADARIVINR</u>	106	O14818	PSMA7
56	<u>QDERTVR-KICALDDHVC</u> <u>MAFAVLTIFIGLTADARVINR</u>	114	Q8TAA3	PSMA8
61	<u>LDSSTVT-HLFKITENIG-----CVMTGMTADSR</u>	113	P60900	PSMA6
57	<u>YDERSVH-KVEPITKHIG</u> <u>LVY-----SGMGPDYRVLVHRARKLAQ</u>	109	P25787	PSMA2
56	<u>LDEVFFSEKIYKL</u> <u>NEDMA-----CSVAGITSDANVL</u>	109	P25789	PSMA4
59	<u>YEEGSNK-RLFNVD</u> <u>RHV-----MAVAGLLADARSLADIAREE</u>	111	P25788	PSMA3
57	<u>AAH---QKKILHVD</u> <u>NHIG-----ISIAGLTADARLLCNFM</u>	107	P25786	PSMA1
	: : : : * : * . . * :			
112	VTQAVSNLALQFGEEDADPGAMSRPFGVALLFGGVD-EKGPQLFHMDPSGTFVQC	170	P28066	PSMA5
107	<u>ITRYIASLQRYTQS-----NGRRPFGISALIVGF</u>	161	O14818	PSMA7
115	<u>ITRFIATLKQKYTQS-----NGRRPFGISALIVGF</u>	169	Q8TAA3	PSMA8
114	<u>LCKRIADISQVYTQN-----AEMRPLGCCMILIGIDE</u>	168	P60900	PSMA6
110	<u>LIVQRVASVMQEYTQS-----GGVRPFGVSL</u>	163	P25787	PSMA2
110	<u>LVTALCDIKQAYTQF-----GKRPFGVSL</u>	164	P25789	PSMA4
112	<u>LADRVAMYVHAYTLY-----SAVRPFGCSF</u>	166	P25788	PSMA3
108	<u>LVSLIGSKTQIPTQR-----YGRRPYGVLLIAGYD-</u>	161	P25786	PSMA1
	: : * * * : . . . : : * : . . .			
171	GSASEGAQSSSQVEVYHKSMTLKEAIKSS--IILKQVME--EKL	225	P28066	PSMA5
162	<u>GRGAKSVREFLEKNYTD</u> <u>EAIETDDLTIKLVICALLEV-----QSGGK</u>	216	O14818	PSMA7
170	<u>GRSAKTVREFLEKNYTD</u> <u>EAIASDSEAIKLAIKALLEV-----QSGGK</u>	224	Q8TAA3	PSMA8
169	<u>GVKQTESTSFLEKKVKKKFDWTFEQTVETAITCLSTVLS--</u>	225	P60900	PSMA6
164	<u>GKNYVNGKTFLEKRYNEDELEDAIHTA--ILTLKESFE--</u>	217	P25787	PSMA2
165	<u>GNNASAAVSMKQDYKEGEMTLKS-ALALAIKVLNKTMD-VSKLS</u>	222	P25789	PSMA4
167	<u>GKARQAakteIEKLQMKEMTCR</u> <u>DIVKEVAKII--YIVHD-EVKDKAFELELSWVGELTN-</u>	222	P25788	PSMA3
162	<u>GARSQSARTYLERHMECFMECNL</u> <u>NELVKHGLRALRETLP</u>	220	P25786	PSMA1
	* : : . . . : : : : . : : : .			
226	--FHMFTKEELEEVIKDI-----	241	P28066	PSMA5
217	--LKILNPEEIEKYVAEIEKEKEENEKKKQKKA--S-----	248	O14818	PSMA7
225	--LKMFSakeVELYVTEIEKEKEEAekKskks--V-----	256	Q8TAA3	PSMA8
226	--KFRILTEAIDAHVALAERD-----	246	P60900	PSMA6
218	--FRRLTPTEVKDYLAATA-----	234	P25787	PSMA2
223	TVIRVLKQKEVEQLIKKHEEEEEAKAEREKKEKE--QKEKDK----	261	P25789	PSMA4
223	--GRHEIVPKD-----IREEAekYAKESLKEE--DESDDDNM--	255	P25788	PSMA3
221	--FTIYDDDDVSPFLEGLEERPQRKAQPAQPADEPAEKADepMEH	263	P25786	PSMA1

FIGURE 4: Alignment of human PSMA subunits 1–8. Protein sequences of the eight proteasome PSMA subunits were aligned using Clustal W. \*, identical residue in all seven subunits; :, conserved amino acids with strongly similar properties; ., conservation between residues of weakly similar properties. Naturally occurring variants are highlighted with grey boxes. Alternatively spliced regions are underlined. Amino acid residue numbers are shown on the left and right of each sequence and the UniProt accession number and gene name of each sequence are shown to the right of each sequence. PSMA8 (PSMA7L) is found only in mammalian testis and is a spermatid/sperm-specific  $\alpha$  subunit [33].

varies in different ethnic populations [60]. Subgroup analysis of the –8C/G polymorphism data showed increased risks of CAD in East Asians, with no significant associations among other ethnic populations. Subgroup analysis also showed increased risks of MI in all populations.

**2.1.3. Type 2 Diabetes Mellitus.** Interestingly, the same –8C > G variant of *PSMA6* gene that was associated with CAD

was found to be associated with T2DM and diabetes-related metabolic traits in two Chinese populations [74]. 73 Caucasian patients with MI and 151 controls genotyped for variants of the *PSMA6* gene revealed no association between *PSMA6* –8C > G and MI [75]. However, 34 diabetic subjects with MI showed a significant association with *PSMA6* –8C > G gene frequency compared to 85 controls [75]. Biopsy specimens taken from the ischemic left ventricle of several



1	-----	0	P49720	PSMB3
1	-----	0	P49721	PSMB2
1	-----ML-----RAGAP---	7	P28065	PSMB9
1	-----MAATLLA-----ARGAGPAPA	16	P28072	PSMB6
1	MAAVSVYAPPVG-----GF-SFDNCRNAV-----LEADFAKRG	33	Q99436	PSMB7
1	<u>MALLDVC</u> <u>GAPRQRPESALPVAGSGRRSD</u> <u>PGHY</u> -SFSMRSP <del>ELALPRG</del> <u>MQ</u> TE-FFQSLG	58	P28062	PSMB8
1	--MEAFLGSRGLWA-----GGPAPGQFYRIPSTPD-----SFMDDPA	35	P28070	PSMB4
1	-----MLSTAMYSAPG	12	P20618	PSMB1
1	--MLKPALEPRG-----GF-SFENCQRNAS-----LERV--LPG	29	P40306	PSMB10
1	<u>MALASVLERP</u> ----- <u>LPVNQR</u> ----- <u>GFF</u> - <u>GLGGR</u> <u>ADLLDLGPGSLSDGLSLAAPG</u>	45	P28074	PSMB5
1	-----MSIMSYNGGAVMAMKGNCAIAADRRFGIQAQMTTDFQKIF	43	P49720	PSMB3
1	-----MEYLIGIQPDYVLVASDRVAASNIVQMKDDHDKMF	36	P49721	PSMB2
8	---TG-----DLPRAGEVHTGTTIMAVEFDGGVVMGSDSRVSAGEAVVNRVDFKLS	55	P28065	PSMB9
17	WGPEA-----FTPDWESREVSTGTTIMAVQFDGGVVLGADSRRTTGSYIANRVTDKLT	69	P28072	PSMB6
34	YK-----LPKVRKTGTTIAGVVYKDGIVLGADTRATEGMVVADKNCSEKIH	78	Q99436	PSMB7
59	GDG-----ERNVQIEMAHGTTLTAFKFQHGVIAAVDSRASAGSYISALRVNKVI	107	P28062	PSMB8
36	SALYR-----GPITRTQNPMVTGTSVLGVKFEGGVVIAADMLGSYGSLARFRNISRIM	88	P28070	PSMB4
13	RDLGMEPHRAAGPLQLRFSPYVFNNGGTILAIAGEDFAIVASDTRLSEGFSIHTRDSPKCY	72	P20618	PSMB1
30	LK-----VPHARKTGTTIAGLVFQDGVILGADTRATNDSVVDKSCSEKIH	74	P40306	PSMB10
46	<u>WGV</u> ----- <u>PEEPGIEMLHGTTT</u> <u>LAFKFRHGVI</u> <u>AAADSRATAGAYIASQT</u> <u>VKKVI</u>	94	P28074	PSMB5
44	PMGDRLYIGLAGLATDVQTVARLKFRLNLYE-LKEGRQIKPYTLMSMVANLLYEKRF--	100	P49720	PSMB3
37	KMSEKILLLCVGEAGDTVQFAEYIQKNVQLYK-MRNGYELSPATAANFTRNLADCLRSR	95	P49721	PSMB2
56	PLHERIYCALSGSAADAQAVADMAAYQLELHG-IELEEPPLVLAAANVVRNISKYRE--	112	P28065	PSMB9
70	PIHDRI FCCRSGSAADTQAVADAVTYQLGFHS-IELNEPLVHTAASLFKEMCYRYRE--	126	P28072	PSMB6
79	FISPNYICCCAGTAADTDMTTQLISSNLELHS-LSTGRLPVVVTANRMLKQMLFRYQG--	135	Q99436	PSMB7
108	EINPYLLGTMSGCAADCQYWERLLAKECRLYY-LRNGERISVSAASKLLSNMMCQYRG-M	165	P28062	PSMB8
89	RVNNSTMLGASGDYADFQYKQVLGQMVIDEELLGDGHSYSPRAIHSWLTRAMYSRRSKM	148	P28070	PSMB4
73	KLTDKTVIGCGSGFGDCLTLTKIEARLKMVK-HSNKNAMTTGAIAAMLSTILYSRRF--	129	P20618	PSMB1
75	FIAPKIYCCGAGVAADAEMTTRMVASKMELHA-LSTGREPRVATVTRILRQTLFRYQG--	131	P40306	PSMB10
95	<u>ENPYLLGT</u> <u>MAGGAADCSF</u> <u>WERLLARQCRIYE</u> -LRNKRISVAAASKLLANMVYQYKG-M	152	P28074	PSMB5
101	GPYYTEPVIAGLDPKTFKPFICSLDLIGCPMVTDDFVVSCTCAEQMYGMCES--LWEPNM	158	P49720	PSMB3
96	TPYHVNLLLAGYDEH-EGPALYYMDYLAA-LAKAPFAAHGYGAFLTSLILDR--YPTPTI	151	P49721	PSMB2
113	DLSAHLMVAGWDQR--EGGQVYG-TLGGMLTRQPPFAIGGSGSTFIYGYVDA--AYKPGM	166	P28065	PSMB9
127	DLMAGII IAGWDQ--EGGQVYSVPMGGMMVRQSF AIGGSGSSYIYGYVDA--TYREGM	181	P28072	PSMB6
136	--YIGAAVLVGGVDVT--GPHLYSIYPHGS-TDKLPYVTMGSGSLAAMAVFED--KFRPDM	189	Q99436	PSMB7
166	--GLSMGSMICGWDDK--GPGLYVDEHGTRLSGNMF--TSGSNTYAYGVMD--GYRPNL	219	P28062	PSMB8
149	NPLWNTMVIIGGYADG--ESFLGYVDMLGVA YEAPSLA-TGYGAYLAQPLLREVLEKQPVL	205	P28070	PSMB4
130	FPYYVYNIIGGLDEEGKGA-VYSFDPVGSY-QRDSFKAGGSAS-----A--MLQPLL	177	P20618	PSMB1
132	--HVGASLIVGGVDLT--GPQLYGVHPHGS-YSRLPFTALGSGQDAALAVLED--RFQPNM	185	P40306	PSMB10
153	--GLSMGTMICGWDDK--GPGLYVVDSEGNRISGATFS-VGSGSVYAYGVMDR--GYSYDL	206	P28074	PSMB5
159	DP-----DHLFETISQAMLNAVDRDASGMGVIVHII EKDKITTRTLK	201	P49720	PSMB3
152	SR-----ERAVELLRKCLEELQKRFILNLPFTFSVRIIDKNGIHDLNLI	194	P49721	PSMB2
167	SP-----EECRRTTDAIALAMSRDGS SGGVIYLVITITAGVDHRVI-	208	P28065	PSMB9
182	TK-----EECLQFTANALALAMERDGS SGGVIRLAAIAESGVERQVL-	223	P28072	PSMB6
190	EE-----EEAKNLVSEAIAGIFNDLGSGSNIDLCVISKNKLDPLR--	230	Q99436	PSMB7
220	SP-----EEAYDLGRRAIAYATHRDSYSGGVNMYHMKEDGWVKVEST	262	P28062	PSMB8
206	SQ-----TEARDLVERCMRVLYYRDARSYNRFQIATVTEKGVIEGPL	248	P28070	PSMB4
178	DNQVGFKNMQNVEHVPLSLDRAMRLVKDVFISAAERDVYTGDAIRICIVTKEGIREETVS	237	P20618	PSMB1
186	TL-----EAAQGLLVEAVTAGILGDLGSGGNVDACVITKTGAKLLR--	226	P40306	PSMB10
207	<u>EV</u> ----- <u>EQAYDLARRAIYQATYRDAYS</u> <u>GGAVNLYHVREDGWIRVSSD</u>	249	P28074	PSMB5
202	ARMD-----	205	P49720	PSMB3
195	SF-----PK--Q--GS-----	201	P49721	PSMB2
209	-----LGNELPKFYDE-----	219	P28065	PSMB9
224	-----LGDQIPKFAVATLPPA-----	239	P28072	PSMB6
231	-----PYTVPNKKGTRLGRYRCEKGT TAVLTEKITPLEIEVLEETVQTMDS	277	Q99436	PSMB7
263	-----DVSDLLHQYREAN-Q-----	276	P28062	PSMB8
249	STETNWDIAHMISGFE-----	264	P28070	PSMB4
238	LRKD-----	241	P20618	PSMB1
227	-----TLSSPTEPVKRSGRYHFVPGTTAVLTQTVKPLTLELVEETVQAMEVE	273	P40306	PSMB10
250	-----NVADLHEKYSGST-P-----	263	P28074	PSMB5

FIGURE 5: Alignment of human PSMB subunits 1–10. Protein sequences of the ten proteasome PSMA subunits were aligned using Clustal W. \*, identical residue in all ten subunits; :, conserved amino acids with strongly similar properties; ., conservation between residues of weakly similar properties. Naturally occurring variants are highlighted with grey boxes. Alternatively spliced regions are underlined. Amino acid residue numbers are shown on the left and right of each sequence and the UniProt accession number and gene name of each sequence are shown to the right of each sequence.

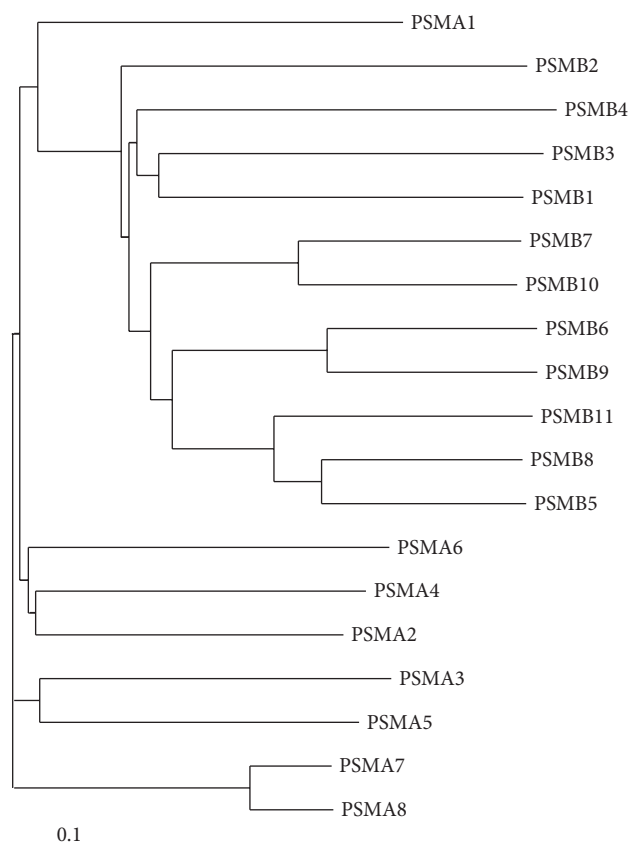


FIGURE 6: Phylogenetic tree of human 20S proteasome subunits. Phylogenetic tree was generated using Clustal W2 phylogeny [88] and image obtained using TreeView [89]. The UniProt accession numbers used for the alignment of proteasome subunits are given in Figures 4 and 5.

patients showed Ub levels and proteasome 20S activity which significantly correlated with plasma glucose levels, with T2DM patients having higher Ub levels and proteasome 20S activity than nondiabetics [75]. This experimental data suggest that the *PSMA6* -8C > G polymorphism contributes to MI susceptibility in T2DM, possibly by upregulation of the UPS. The *PSMA6* -8C > G polymorphism was also reported to be associated with lower survival rates in multiple myeloma patients [93].

**2.1.4. Graves' Disease.** Graves' disease is an autoimmune thyroid disease characterized by hyperthyroidism due to circulating autoantibodies. It is one of the most common thyroid problems and several immune and thyroid related genes appear to influence susceptibility to Graves' disease [94]. A 270 kb chromosome region (14q13.2-14q13) containing *PSMA6* was analyzed for polymorphisms and associations of five microsatellite repeats in 50 Latvian patients with Graves' disease and 116 controls with Graves' disease [87]. Some particular alleles of HSMS006 and HSMS801 (microsatellite polymorphisms) were found more often while some alleles of HSMS006 were found less frequently in Graves' patients when compared to healthy controls. The HSMS602 allele

was found in Graves' patients but not in healthy controls [87]. Further analysis is needed to confirm the importance of *PSMA6* in Graves' disease.

**2.1.5. Psoriasis.** Several psoriasis susceptibility loci have now been detected [95]. A meta-analysis of two GWA studies involving 1,831 cases and 2,546 controls gave 102 potential loci. A three-stage replication study using 4,064 cases and 4,685 controls from Michigan, Toronto, Newfoundland, and Germany found three genomic regions, including one that contains *PSMA6* and *NFKB1A* (rs12586317) that showed psoriasis susceptibility. The SNP rs12586317 was strongly associated with the subphenotypes of psoriatic arthritis and purely cutaneous psoriasis [95].

**2.2. PSMA7.** The proteasome gene *PSMA7* codes for  $\alpha 4$ , a 248 residue protein that is similar to *PSMA6* in structure. It is also posttranslationally modified by phosphorylation, glycosylation, and lysine acetylation [60, 84, 96]. Phosphorylation of  $\alpha 4$  at Tyr 153 impaired G1/S transition and S/G2 progression in cells, suggesting that tyrosine phosphorylation of the  $\alpha 4$  proteasome subunit is important in intracellular regulatory control [96].

**2.2.1. Intellectual Disability.** Sequencing the coding regions of more than 21,000 genes from 100 patients with an IQ below 50 and their unaffected parents identified 79 *de novo* mutations (affecting 77 genes) in 53 of 100 patients [45]. A *de novo* heterozygous 335C-A transversion in *PSMA7*, resulting in an A112D mutation, was identified in a male patient with severe intellectual disability [45], suggesting that *PSMA7* may be a candidate intellectual-disability gene.

### 3. Immunoproteasome Mutations and Polymorphisms

Specialized proteasomes called immunoproteasomes (Figure 3) are capable of cleaving substrates to generate short peptide fragments that are recognized as antigens in lymphocytes [23, 29, 97]. These antigens are presented on the surface of these cells (through the MHC complex) and play an important role in the cell's ability to mount a specific immune response [97]. When an infection occurs, the hormone interferon is excreted locally, resulting in gamma-interferon inducible beta subunits which replace the constitutive beta subunits. In many eukaryotic immunoproteasomes the 19S complex is replaced by another complex, the PA28 (or 11S) complex. The cytosolic PA28 complex is composed of a six-member ring of PA28 $\alpha$  and PA28 $\beta$  subunits which are products of *PSME1* and *PSME2* genes, respectively. Nuclear immunoproteasomes contain a PA28 $\gamma$  complex (*PSME3* gene). The PA28 complexes (caps) are significantly smaller than the 19S complexes but are more efficient at generating antigen peptides. They degrade proteins in an ATP-independent manner unlike the 26S proteasome [97, 98]. Two immunoproteasome genes, *PSMB8* and *PSMB9*, have been shown to be associated with human diseases. Surprisingly, no polymorphisms in the genes connected to the constitutive proteolytic activities of the

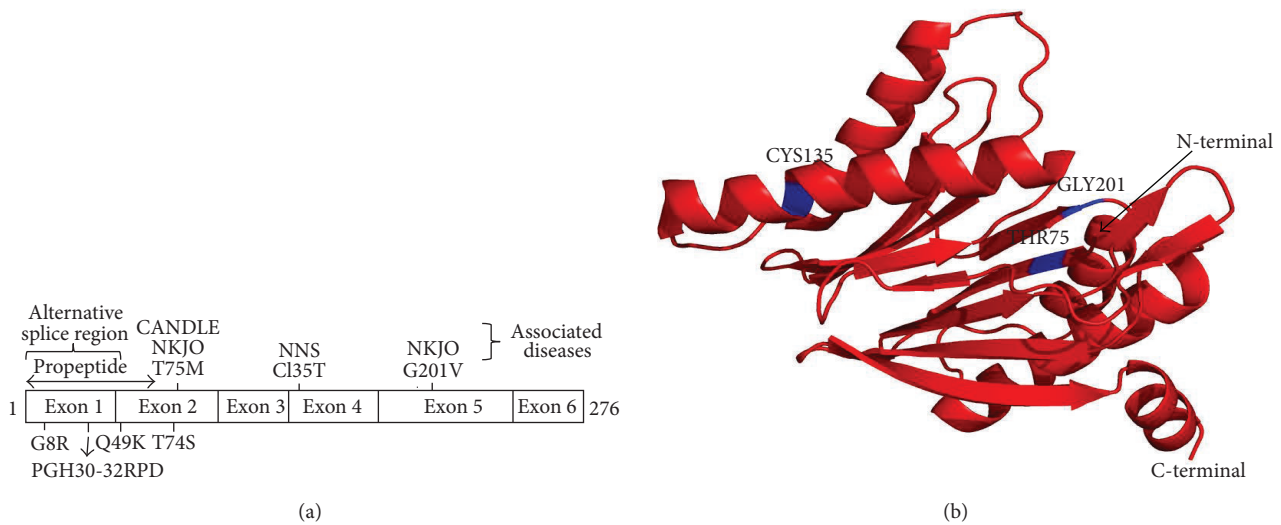


FIGURE 7: Schematic diagram of PSMB8 showing the location of known polymorphisms. (a) Diagram of PSMB8 showing exon organization (drawn to relative scale), location of alternative spliced region, propeptide region that is removed in the mature form of the protein, location of disease causing polymorphisms, and location of other known polymorphisms. (b) Tertiary structure of  $\beta 5i$  (PSMB8) showing polymorphisms (shown in blue) associated with diseases. Structure created using PyMol (<http://pymol.org/>).

proteasome (PSMB5, PSMB6, and PSMB7) have been found to be associated with disease. It is possible that polymorphisms in PSMB5, PSMB6, or PSMB7 that result in decreased proteasome activity may be severe enough that they cause embryonic lethality.

**3.1. PSMB8.** PSMB8 (proteasome subunit  $\beta$  type 8) gene expression is induced by interferon- $\gamma$  (IFN- $\gamma$ ), resulting in the upregulation of the protein product of this gene,  $\beta 5i$ , which replaces the constitutive catalytic subunit  $\beta 5$  (PSMB5) [99]. The human  $\beta 5i$  is expressed as a 276 residue protein which requires the proteolytic removal of 72 residues to generate a mature subunit [100]. Although the  $\beta 5i$  propeptide is not essential for incorporation into the 20S proteasome, presence of this sequence increases the efficiency of  $\beta 5i$  incorporation and proteasome maturation [101]. Two alternative spliced forms of human  $\beta 5i$  have been detected, but both forms would result in the same mature protein, as the alternative splicing occurs in the propeptide which is missing in the mature form of  $\beta 5i$ . The replacement of  $\beta 5$  by  $\beta 5i$  increases the ability of the immunoproteasome to cleave peptides after hydrophobic and basic residues. Mice lacking the PSMB8 gene had reduced levels of MHC class I cell-surface expression and inefficiently presented the endogenous antigen HY [102]. A selective inhibitor of  $\beta 5i$ , ONX-0914 (previously referred to as PR-957), blocked presentation of MHC class I-restricted antigens *in vitro* in splenocytes and *in vivo* in mice [103]. In mouse models, inhibition of  $\beta 5i$  reversed signs of rheumatoid arthritis and reduced cellular infiltration, cytokine production, and autoantibody levels, suggesting that  $\beta 5i$  has an important role in regulating pathogenic immune responses [103]. PSMB8 has recently been shown to have a role in cytokine production [103]. ONX-0914 blocked the production of IL-6, IL-23, and TNF- $\alpha$  by

~50% or greater [103]. ONX-0914 also ameliorated disease in two mouse models of arthritis [103].

Genetic variants of PSMB8 are associated with the development of many diseases, including viral infection, autoimmune disease, and malignant tumors. Figure 7 shows a schematic diagram of the well-annotated polymorphisms as well as the exon organization of PSMB8. The structure of the protein product of PSMB8 showing the location of the three residues associated with diseases is also shown in Figure 7. The residues that are mutated by the disease-associated polymorphisms in PSMB8 are highly conserved from zebrafish to man (Figure 8).

**3.1.1. JMP Syndrome.** JMP syndrome (autosomal-recessive autoinflammatory syndrome characterized by joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy) patients show hepatosplenomegaly and hypergammaglobulinemia as well as lipodystrophy of the arms, face, and thorax. Using genome-wide homozygosity mapping, a homozygous missense mutation (c.224C > T, Thr75Met) in the proteasome gene PSMB8 that encodes the  $\beta 5i$  (LMP7) subunit was detected in two pedigrees from Portugal and Mexico with JMP syndrome [54]. Segregation of this mutation in other members of the pedigrees occurred in an autosomal-recessive fashion. Measurement of proteasome activity in the cell lysates of Epstein-Barr virus-transformed lymphoblasts from a control subject and an affected (T75 M) patient showed reduced chymotrypsin-like activity, but similar trypsin-like and caspase-like activity in the affected patient relative to the control subject. Serum from two affected patients showed 2.8- to 3.5-fold, 1.6- to >9-fold, and 7- to 19-fold increased levels of interferon  $\gamma$ , IL-8, and IL-6 respectively. These results and other results from these patients, such as increased serum  $\gamma$  globulins and

1	MALLDLCGAPRGQRPEWAAVDAGSGLRSDPGHYSFSVQAPELALPRGMQPTFLRSFGDD	60	P28064	Rat
1	MALLDVCGAPGGQRGDWA VPLAGSRQSRSDPGHYGFSLSRSPALPRGMQPTFFRSLGGN	60	Q3T112	Bovine
1	MALLEVCGAPRGLRKACAVPALGSQLRSDPGHYSFSLSRAPELAVPRGMQPTFFQSLGEN	60	Q5W416	Dog
1	MALLDLCGAARQRPWEAALDAGSGGRSDPGHYSFSAQAPELALPRGMQPTAFLRSFGDD	60	P28063	Mouse
1	MALLDVCGAPRGQRPEALPVAGSGRRSDPGHYSFSMRSPALPRGMQPTFFQSLGGD	60	P28062	Human
1	MALLDVSQYKYNASQFGF---KQTLDRSNHYSFGTKCFEAVPVGVDPKFLKSCS--	55	Q6NZ73	Zebrafish
1	MALLEVCGAPRGQRKDCAFSTLGSQLRSDPGHYSFSLSRSPFALPRGMQPTFFQSHGGN	60	M3XF92	Cat
1	MALLTICGPTQS--QDWRMPLCGGTI--SPTVPFQVYNTLAVPPGYQPAKFLEHLE-E	54	A8E5T8	Western frog
1	MALQQVCGPSGWKDCCLSSP-----PSLSGRSPFSSGTLDFEVPPGLQPSAFLKSLVGN	54	A7X5P0	Platypus
1	MALLTMCGPTQS--HDWRMPLYGGTI--SPTIPFRVCMTELAVPPGYQPAKFLQHLE-E	54	Q91787	African frog
1	MALLEVCGASPRQRADWALPAAGSGHRSVPGHYSFSMRSPALPRGMQPTDFLQSLGGN	60	F6Z8V2	Horse
	*** :.* * : : * * : * : *			
61	QERKVQIEMAHGTTTLAFKFQHGVI VAVDSRASAGSYIATIRVNKVEINPYLLGTMSGC	120	P28064	Rat
61	GESKVQIEMAHGTTTLAFKFQHGVI VAVDSRASAGNYIATLKNKVEINPYLLGTMSGC	120	Q3T112	Bovine
61	GEKNIRKEMVHGTTTLAFKFQHGVI VAVDSRATAGNYISSRVNKNVEINPSSLGTMSGC	120	Q5W416	Dog
61	QERNVQIEMAHGTTTLAFKFQHGVI VAVDSRATAGSYISSLRMNKNVEINPYLLGTMSGC	120	P28063	Mouse
61	GERNVQIEMAHGTTTLAFKFQHGVI VAVDSRASAGSYISALRVNKNVEINPYLLGTMSGC	120	P28062	Human
56	CEDGVCIDLNHGTTTLAFKFQHGVI VAVDSRASAGKYIDSKEANKVEINPYLLGTMSGC	115	Q6NZ73	Zebrafish
61	GERNVQIEMAHGTTTLAFKFQHGVI VAVDSRASAGTYIATLRNKNVEINPYLLGTMSGC	120	M3XF92	Cat
55	GVDDVKIEPWHGTTTLAFKFQHGVI VAVDSRASAGSYISNVKFNKNVEINPYLLGTMSGC	114	A8E5T8	Western frog
55	RDDRQIKLFKGTTLAFNFKHGVI VAVDSRATAGNYICSMQFNKNVEINPQLLGTMSGC	114	A7X5P0	Platypus
55	GVDDVKIEPWHGTTTLAFKFQHGVI VAVDSRASAGSYISTIKFNKNVEINPYLLGTMSGC	114	Q91787	African frog
61	GEENVQIEMAHGTTTLAFKFQHGVI VAVDSRASAGSYIATLRVNKNVEINPYLLGTMSGC	120	F6Z8V2	Horse
	: . :*****:*.***:*****:*.** ***** *****.			
121	AADCQYWERLLAKECRLYLRNGERISVSAASKLLSNMMLQYRGMGLSMGSMICGWDKKG	180	P28064	Rat
121	AADCYWERLLAKECRLYLRNGERISVSAASKLLSNMMCQYRGMGLSMGSMICGWDKKG	180	Q3T112	Bovine
121	AADCQYWERLLAKECRLYLRNGERISVSAASKLLSNMVYQYRGMGLSMGSMICGWDKKG	180	Q5W416	Dog
121	AADCQYWERLLAKECRLYLRNGERISVSAASKLLSNMMLQYRGMGLSMGSMICGWDKKG	180	P28063	Mouse
121	AADCQYWERLLAKECRLYLRNGERISVSAASKLLSNMMCQYRGMGLSMGSMICGWDKKG	180	P28062	Human
116	AADCQYWERLLAKECRLYKLNRKQIRISVSAASKLLSNMMLGYRGMGLSMGSMICGWDKQG	175	Q6NZ73	Zebrafish
121	AADCQYWERLLAKECRLYLRNGERISVSAASKLLSNMVYQYRGMGLSMGSMICGWDKKG	180	M3XF92	Cat
115	AADCQYWERVLAKECRLYQLRNNRSISVSAASKLMCNMMLQYRGTGLSVGSMICGWDKKG	174	A8E5T8	Western frog
115	AADCQYWERLLAKECRLYHLRNGERISVSAASKLLSNMMCQYRGMGLSMGSMICGWDKKG	174	A7X5P0	Platypus
115	AADCQYWERLLAKECRLYQLRNNRSISVSAASKLMCNMMLQYRGTGLSVGSMICGWDKKG	174	Q91787	African frog
121	AADCQYWERLLAKECRLYLRNGERISVSAASKLLSNMMCQYRGMGLSMGSMICGWDKKG	180	F6Z8V2	Horse
	**** *:***** ** .*****:*.** ***:*****.*			
181	PGLYYVDNNGTRLGSGQMFSTGSGNTYAYGVMDSGYRQDLSPEEAYDLARRAIVYATHRDS	240	P28064	Rat
181	PGLYYVDENGTRLGSGNMFSTGSGNSHAYGVMDSGYRDLSEIEAYDLGRRRAIVHATHRDS	240	Q3T112	Bovine
181	PGLYYVDQNGTRLGSGNMFSTGSGSTYAYGVMDSGYQPSLSPPEEAYELGRRRAITYATHRDS	240	Q5W416	Dog
181	PGLYYVDNNGTRLGSGQMFSTGSGNTYAYGVMDSGYRQDLSPEEAYDLGRRRAIYATHRDN	240	P28063	Mouse
181	PGLYYVDEHNGTRLGSGNMFSTGSGNTYAYGVMDSGYRPNLSPEEAYDLGRRRAIYATHRDS	240	P28062	Human
176	PGLYYVDNNGTRLGSGRMFSTGCGNSYAYGVVDSGYREDMTVEEAYELGRRRAIYATHRDA	235	Q6NZ73	Zebrafish
181	PGLYYVNSDGTRLGSGNMFSTGSGNTYAYGVMDSGYRQDLSPEEAYELGRRRAIVYATHRDS	240	M3XF92	Cat
175	PGLYYVDNNGTRLGCDIFSTGSGNSYAYGVMDSGYRDLTPEEAYDLGRRRAISYATHRDA	234	A8E5T8	Western frog
175	PGLYYVDEDDGRLSGLMFSTGSGRNYAYGVMDSGYRDLSPPEEAYDLGRRRAIVHATHRDG	234	A7X5P0	Platypus
175	PGLYYVDNNGTRLGCDIFSTGSGNSYAYGVMDSGYRDLTPEEAYDLGRRRAISYATHRDA	234	Q91787	African frog
181	PGLYYVDENGTRLGSGNMFSTGSGNSYAYGVMDSGYRNLSIEEAYDLGRRRAIVHATHRDS	240	F6Z8V2	Horse
	*****:..*.** *:***.* :*****:***: : : *****.*.* :*****			
241	YSGGVVNMYHMKKDGWVKVESTDVSDLLHKYREATL-	276	P28064	Rat
241	YSGGVVNMYHMKEDGWVKVESTDVSDLMHQYREASQ-	276	Q3T112	Bovine
241	YSGGIINMYHMKEDGWVKVESTDVNELLHQYQEANQ-	276	Q5W416	Dog
241	YSGGVVNMYHMKEDGWVKVESDVSDDLKYKGEAAL-	276	P28063	Mouse
241	YSGGVVNMYHMKEDGWVKVESTDVSDLLHQYREANQ-	276	P28062	Human
236	YSGGVVNLYHMQEDGWIKVCKEDVSELIHRYKKGMF-	271	Q6NZ73	Zebrafish
241	YSGGVVNMYHMKEDGWVKLESTDVSDLLHRYREASQ-	276	M3XF92	Cat
235	YSGGFVNLYHMKEDGWVKIGQFDVNDLLHKYTEEKNM	271	A8E5T8	Western frog
235	YSGGMVNMYHMKQDGIKVEQTDVNDLLSTLEARV-	270	A7X5P0	Platypus
235	YSGGCVNLYHMKEDGWVKIGQFDVSDLLHKFTTEKNM	271	Q91787	African frog
241	YSGGVVNMYHMKEDGWVKVESTDVSDLLHQYREASH-	276	F6Z8V2	Horse
	**** :*:***:***: : . ***:** :			

FIGURE 8: Sequence alignment of human PSMB8 from different animals. Protein sequences of *Rattus norvegicus* (rat), *Bos taurus* (bovine), *Canis familiaris* (dog), *Mus musculus* (mouse), *Homo sapiens* (human), *Danio rerio* (Zebrafish), *Felis catus* (Cat), *Xenopus tropicalis* (western clawed frog), *Ornithorhynchus anatinus* (Duckbill platypus), *Xenopus laevis* (African clawed frog), and *Equus caballus* (Horse) PSMB8 were aligned using Clustal W. \*, identical residue in all six subunits; :, conserved amino acids with strongly similar properties; ., conservation between residues of weakly similar properties. Naturally occurring variants are highlighted with grey boxes. Alternatively spliced regions are underlined. Amino acid residue numbers are shown on the left and right of each sequence and the UniProt accession number is also shown.



erythrocyte sedimentation rate without elevation in other cytokines such as IL-1 and TNF- $\alpha$ , suggest significant ongoing inflammation and a potentially unique biomarker signature in JMP syndrome patients.

**3.1.2. Nakajo-Nishimura Syndrome.** Nakajo-Nishimura syndrome (NNS) was first reported by Nakajo in 1939 [104]. NNS is a rare, distinct inflammatory, and wasting disease which usually begins in early infancy and has only been reported in Japanese patients [105]. Clinical features of this disease include elongated and thickened fingers, hereditary lipomuscular atrophy with joint contractures, periodic high fever, hyper- $\gamma$ -globulinemia nodular erythema, and myositis [106, 107]. Extracts separated by glycerol gradient centrifugation from immortalized lymphoblastoid cell lines obtained from an NNS patient, his heterozygous parent, and a healthy control showed that all three proteolytic activities of the proteasome were decreased in the NNS patient relative to the healthy control. Due to the low number of samples investigated, the results should be viewed with caution but do suggest that the G210V mutation is associated with decreased immunoproteasome activity. NNS cells also show an accumulation of immature 20S proteasome precursors before incorporation of  $\beta$ 5i into the complex. In silico modeling of the G210V suggests that this assembly defect could be due to the proximity of  $\beta$ 5i,  $\beta$ 4, and  $\beta$ 6 next to each other resulting in conformation changes in both Thr73 and Lys105 which are part of the catalytic center of PSMB8. Interestingly, some of the G210V mutant  $\beta$ 5i subunits incorporated into the mature proteasome appeared to be insufficiently cleaved. These results suggest that the G210V mutation affects both  $\beta$ 5i catalytic activity and assembly of the 20S proteasome. A polymorphism in PSMB8 resulting in a Q49K amino acid change in  $\beta$ 5i was found to be associated with juvenile rheumatoid arthritis [108]. Some of the features of juvenile rheumatoid arthritis are similar to NNS.

**3.1.3. Candle Syndrome.** Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE syndrome) is a recently described autoinflammatory syndrome [77]. Autoinflammatory diseases differ from autoimmune diseases in that they are primarily a result of alterations in the innate immune system instead of perturbations in adaptive immunity [109]. Patients with CANDLE syndrome typically show recurrent fevers, hypochromic or normocytic anemia, delayed physical development, and variable clinical features including acanthosis nigricans (skin hyperpigmentation), alopecia areata (spot baldness), and hypertrichosis (werewolf syndrome) [109, 110]. A recent genome-wide analysis of nine affected patients in eight families suggests that mutations in PSMB8 may be the molecular basis of CANDLE syndrome [55]. Four patients were homozygous and two were heterozygous for a missense mutation (c.224C > T), two patients were homozygous for a nonsense mutation in PSMB8 (c.405C > A), and one patient showed no mutation. None of these sequence changes were observed in chromosomes from 750 healthy controls. Only two of the four patients with the same mutation shared the same haplotype, indicating a mutational hot spot.

**3.1.4. Bacterial Infection.** *Mycobacterium tuberculosis* (*M. tuberculosis*) infection is a common bacterial infection that is the leading cause of morbidity and mortality compared to all other infectious agents [111]. Extrapulmonary tuberculosis, which is common in the intestinal tract, bones, kidney, lymph gland, skin, and other organs, occurs in 5–20% of all tuberculosis cases and is increasing in both developed and developing countries [112]. Using PCR-based restriction digest, sequencing of digests, and logistic regression analysis, a study involving 168 Chinese patients with intestinal tuberculosis and 235 normal controls identified a polymorphism in PSMB8 (Q145K) which were found to be associated with intestinal *M. tuberculosis* infection [78]. *M. tuberculosis* antigenic peptides are produced by the immunoproteasome and subsequently presented on the cell surface by the MHC-I molecule resulting in CD8+ cytotoxic T lymphocytes eliminating *M. tuberculosis* infected cells. Mice lacking the three immunoproteasome catalytic subunits showed defects in presenting several major histocompatibility complex (MHC) class I epitopes in dendritic cells [113]. During viral infection *in vivo*, the MHC class I-presented peptides in immunoproteasome-deficient animals were significantly reduced compared with wild-type mice, whereas presentation of MHC class II peptides was unaffected. These reductions in MHC class I-presented peptides and changes in the type of class I-presented peptides caused transplant rejection of wild-type cells in mutant mice [113].

**3.1.5. Cancer.** A high risk of colon cancer was associated with a LMP7-K/Q genotype (PSMB8) while a low risk was associated with the LMP7-Q/Q genotype in an investigation of 112 colorectal carcinoma patients and 62 control patients [79]. Stimulation of colon carcinoma cell lines with interferon (IFN)- $\gamma$  exhibited a 10-fold increase in LMP7-Q transcript amounts, but only 3.8-fold increase in LMP7-K [79]. The LMP7-K allele showed reduced transcript stability compared with LMP7-Q. Overall, the LMP7-K allele seems to reduce the immunoproteasome formation which results in reduced peptide processing and reduced peptide-HLA presentation [79]. Peptide-HLA presentation is a crucial factor in the immune response against cancer. Immunoproteasomes generate immunogenic tumor peptides which are important for the destruction of cancer cells by cytotoxic T lymphocytes.

**3.1.6. Ankylosing Spondylitis.** Ankylosing spondylitis (AS) is an inflammatory rheumatic disease which affects men more often than women and is strongly associated with human leucocyte antigen (HLA)-B27 and with the fusion of the spine vertebrae [114]. Inflammation of the joints is common in AS but other parts of the body, such as eyes and bowels, can also show inflammation. The first study to suggest that the PSMB8 gene was associated with AS involved 57 AS patients and 102 matched random controls [80]. This investigation found that the HLA-B27 polymorphism in PSMB9 and LMP7-Q/Q (PSMB8) confers a higher relative risk than with HLA-B27 alone. A significant association was observed between the LMP7-Q/Q genotype and AS.

**3.1.7. Type 1 Diabetes Mellitus.** A genomic polymorphism (G/T-37360) in *PSMB8* was strongly associated with type 1 diabetes mellitus (T1DM) in an investigation of 198 unrelated T1DM Caucasian patients and 192 normal Caucasian controls from the southeastern United States [81]. The R/H-60 polymorphism in *PSMB8* was found to be associated with T1DM only in subjects containing an HLA DR4-DQB1\*0302 haplotype. However, results from this same study suggest that *PSMB8* genes have independent effects on T1DM susceptibility [81].

Some of the clinical features of *PSMB8* mutations may be due to the role of *PSMB8* in autophagy as *PSMB8* seems to play a key role in apoptosis. IFN- $\gamma$  causes increased sensitivity to apoptosis in atherosclerotic lesions. IFN- $\gamma$  sensitized cells from the fibrous cap of human atherosclerotic lesions showed reduced Mcl-1, phospho-Bcl-2 (S70), and phospho-Bcl-X(L) (S62) protein levels. Knockdown of *PSMB8* with siRNA protected the antiapoptotic protein Mcl-1 from degradation [115]. These results suggest that the immunoproteasome may be a key link between inflammatory factors and the control of vascular cell apoptosis [115].

**3.2. *PSMB9*.** Like *PSMB8* expression, *PSMB9* gene expression is induced by IFN- $\gamma$ , resulting in the upregulation of the protein product of this gene  $\beta$ li, which replaces the constitutive catalytic subunit  $\beta$ 1 (*PSMB6*) [99]. The human  $\beta$ li is expressed as a 219 residue protein which requires the proteolytic removal of 20 residues to generate a mature subunit. Although two alternative transcripts which encode different isoforms have been reported (Figure 5), the alternative splicing occurs in the region of  $\beta$ li (propeptide) that is removed in the mature form, resulting in the same mature protein. Upregulation of MHC-linked  $\beta$ li and  $\beta$ 5i subunits amplifies specific endopeptidase activities of the proteasome resulting in the increased production of peptides which terminate almost exclusively with hydrophobic or basic residues, such as those found on MHC class I molecules [99, 116].

$\beta$ li-deficient mice, generated by replacing a 800 bp region of the *PSMB9* gene with a neomycin resistance gene in embryonic stem cells, were viable, and healthy with no gross anatomical abnormalities [117]. Purified proteasomes from the spleen and liver of  $\beta$ li-deficient mice showed lower peptidase activity against hydrophobic and basic substrates (but not acidic substrates) when compared to purified proteasomes from wild-type tissues. Antigen-presenting cells from  $\beta$ li-deficient mice displayed reduced ability to stimulate a T-cell hybridoma specific for a nucleoprotein envelope antigen of an influenza A virus [117].  $\beta$ li-deficient mice also showed only 60%–70% of wild-type levels of CD8-positive T lymphocytes and generated fewer influenza nucleoprotein-specific cytotoxic T lymphocyte precursors. Hence  $\beta$ li is important in antigen processing of MHC class I-restricted antigens.

**3.2.1. Graves' Disease.** Several investigations demonstrated potential associations between codon 60 R/H polymorphism in *PSMB9* (p.60R > H; c.179G > A; rs17587) and increased susceptibility to various diseases. This *PSMB9* genetic R/H polymorphism at codon 60 had H allele frequencies of 1.1%

to 34%, depending on ethnic group [118, 119]. DNA from 306 Caucasian patients with Graves' disease and 364 Caucasian control subjects were investigated for the distribution of an R/H polymorphism in the *PSMB9* gene and a G/T polymorphism in the *PSMB8* gene [82]. The R allele and the RH genotype were increased in subjects with Graves' disease when compared with control subjects. Independently, DNA from 129 families, including parents, an affected sibling with Graves' disease, and an unaffected sibling, were investigated. No preferential allelic transmission occurred from heterozygote parents to offspring at either locus, suggesting that the association of the R/H polymorphism at codon 60 of *PSMB9* with Graves' disease is due to linkage disequilibrium with the associated HLA haplotype [82].

Mishto et al., 2006, [120] found that the codon 60 R/H polymorphism results in a decreased chymotrypsin-like proteasome activity in the aged brain, while recombinant peptides mimicking endogenous substrates showed no differences in the substrate hydrolysis profiles between the codon 60 genotypes [121]. Using fluorogenic substrates that are hydrolyzed selectively by  $\beta$ li, measurement of  $\beta$ li catalytic activity showed that the codon 60 R/H polymorphism did not alter the activity of  $\beta$ li among the cancer cell lines tested [122]. Western blotting showed that the levels of  $\beta$ li were highly elevated in clinical colon cancer tissues compared to the paired nonmalignant colonic tissues. These effects all suggest inconsistent results regarding the influence of this polymorphism on proteasome activity.

**3.2.2. Colorectal Cancer.** Genotyping of 1467 SNPs (in 871 candidate cancer genes) in 2575 Caucasian colorectal cancer patients and 2707 controls indicated an association with 44 SNPs and colorectal cancer [123]. One of these SNPs, rs241419 (V32I) in *PSMB9*, showed a significant association with an increased risk of colorectal cancer. However, validation of rs241419 association with colorectal cancer was not carried out using kin-cohort analysis of first-degree relatives as was carried out for some other SNPs validated [123].

**3.2.3. Ankylosing Spondylitis.** 193 unrelated Caucasians and 49 Chinese B27 individuals with AS were investigated to determine *PSMB9* gene influence on disease susceptibility in HLA-B27 individuals with AS [83]. HLA-B27 typing showed the involvement of the *PSMB9* gene in the expression of AS in B27 individuals. The LMP2BB genotype (*PSMB9*) was investigated in 546 AS patients (41 Caucasians and 17 Mexican) and 4352 controls. The LMP2BB genotype was significantly decreased in Caucasian and Mexican AS patients compared to random Mexican and Caucasian controls, respectively [124].

**3.2.4. 19S Proteasome Mutations and Polymorphisms.** Alignment of human *PSMC* (Figure 9) and human *PSMD* (Figure 10) shows the relatedness of the 19S proteasome subunits. *PSMF1* shares some homology with *PSMD12* (approximately 40%) while *PSMD8* shares homology with *PSME4* (approximately 40%) (Figure 11). Limited data suggests that four 19S genes, *PSMD3*, *PSMD7*, *PSMD13*, and *PSMD14*, may be associated with human diseases.

1	MGQSQSGGHGPGGGKKDDKDKKKKYEPVPTRVGKKKKKTGPDAA-----KLPL	51	P62191	PSMC1
1	-----	0	Q75L23	PSMC2
1	-----MNLPLNIESPVT-----RQEKMATVW	21	P17980	PSMC3
1	-----MAL	3	P62195	PSMC5
1	-----M	1	P62333	PSMC6
1	-----MEEIGILVEK--AQDEIPALSVSRPQTGLSF	29	P43686	PSMC4
	: : .			
52	VTPHTQ----CRLKLLKLERIK-----DYLMEEEFIRNQEQMKPLEEKQEEERSKV-	99	P62191	PSMC1
1	-----LDEGDIA-----LLKTYG--QSTYSR---QIKQVEDDIQQLLKKINELTGIKE	43	Q75L23	PSMC2
22	DEAEQDGIGEEVLKMSTEEIIQTRRLDSEIKIMKSEVLRVTHELQAMKDKIKENSEKIK	81	P17980	PSMC3
4	DGPEQMELEEGKAG-----SGLRQYY--LSKIEELQLIVNDKSQNLRLQAQRNELNAKV-	56	P62195	PSMC5
2	ADPR-----D---KALQDYR--KKLL---EHKEIDGRKLKELREQLKELTKQY-	40	P62333	PSMC6
30	LGPEPEDLEDLYSRYYKLQQL-----EFLEVQEEYIKDEQK--NLKKEFLHAQEEV-	79	P43686	PSMC4
	. . . : : .			
100	-----DDLRGTP-----MSVGTLEEIIIDNHAIVSTSVGSEHYVS	134	P62191	PSMC1
44	SDTGL--APPALWDLAADKQTLQSEQPLQVARCTKIINADSEDPKYIINVKQFAKFVVD	100	Q75L23	PSMC2
82	VNKTLPYLVSNVIELLDVDPNDQEDG-----ANIDLDSQRKKGCAVIKTSTRQTYFLP	135	P17980	PSMC3
57	-----RLLEELQLLQEQG-----SYVGEVVRAMDKKKVLVKVHPEGKFVVD	98	P62195	PSMC5
41	-----EKSENDLKALQSVG-----QIVGEVLKQLTEEFKIVKATNGPRYVVG	82	P62333	PSMC6
80	-----KRIQSIP-----LVIGQFLEAVDQNTAIVGSTTGSNYYVR	114	P43686	PSMC4
	: : * : : * . * : : : . * : * . *			
135	ILSFVDKDLLEPGCSVLLNHKVHAVIGVLMDDTDLVTVMKVEKAPQETYADIGGLDNQI	194	P62191	PSMC1
101	LSDQVAPTDEEGMRVGVDRNKYQIHIPLPPKIDPTVTMMQVEEKPDVTYSDVGGCKEQI	160	Q75L23	PSMC2
136	VIGLVDAEKLKPGDLGVGNKDSYLILETLPTEYDSRVKAMEVDERPTEQYSDIGGLDKQI	195	P17980	PSMC3
99	VDKNIDINDVTPNCRVALRNDSTYTLHKILPNKVDPLVSLMMVEKVPDSTYEMIGGLDKQI	158	P62195	PSMC5
83	CRRQLDKSKLPGTRVALDMTTLTMYRLPREVDPLVYNMSHEDPGNVSYSEIGGLSEQI	142	P62333	PSMC6
115	ILSTIDRELLKPNASVALHKHSNALVDVLPPEADSSIMMLTSDQKPDVMDYADIGMDIQK	174	P43686	PSMC4
	. : * : * : . * : : * * * : : * * * : : * : * : .			
195	QEIKESVELPLTHPEYYEEMGIKPPKGVILYGGPGTGKTLAKAVANQTSATFLRVVGSE	254	P62191	PSMC1
161	EKLREVVETPLLHPRFVNLGIEPPKGVLLFGPPGTGKTLACAVANRTDACFIRVIGSE	220	Q75L23	PSMC2
196	QELVEAIVLPMNHKEKFENLGIQPPKGVLMYGGPGTGKTLARACAAQTATFLKLAGPQ	255	P17980	PSMC3
159	KEIKEVIELPVKHPELFEALGIAQPKGVLYGGPGTGKTLARAVAHHTDCTFIRVSGSE	218	P62195	PSMC5
143	RELREVELPLTNPELFRVGIIIPKGCILYGGPGTGKTLARAVASQLDCNFLKVVSSS	202	P62333	PSMC6
175	QEVREAVELPLTHFELYKQIGIDPPRGVLMYGGPGCGKTMKAKAVAHHTAAAFIRVVGSE	234	P43686	PSMC4
	: : : : * : : * . . : : * * * : * . . : : * : * : : * : *			
255	LIQKYLGDGPKLVRELFRVAEEHAPSIVFIDEIDAIGTKRYDSNNGGEREIQRMTLELLN	314	P62191	PSMC1
221	LVQKYVGEARMVRELFEARTKKACLIFFDEIDAIGGARFDDGAGGDNEVQRTMLELIN	280	Q75L23	PSMC2
256	LVQMFIDGAKLVDAFALAKEKAPSIIFIDEIDAIGTKRFDEKAGDREVQRTMLELLN	315	P17980	PSMC3
219	LVQKFIGEGARMVRELFMAREHAPSIIFMDEIDSIGSSRLGSGSGDSEVQRTMLELLN	278	P62195	PSMC5
203	IVDKYIGESARLIREFMNYARDHQPCIIIFMDEIDAIGGRFSEGTSADEIQRITMELLN	262	P62333	PSMC6
235	FVQKYLGEGRPMVRDVFRLAKENAPAIIFIDEIDAIAIKRFDAQTGADREVQRIILELLN	294	P43686	PSMC4
	* : * * : : * : * * : * * * : * : . : * : . : .			
315	QLDGFDSRGDVVKVIMATNRIETLDPALIRPGRIDRKIEFPLPDEKTKKRIFQIHTSRMTL	374	P62191	PSMC1
281	QLDGFDPGRNIKVMATNRPDTLDPALMRPGRDLDRKIEFSLPDLEGRTHIFKIHARMSV	340	Q75L23	PSMC2
316	QLDGFQPNQVQVIAATNRVDILDPALLRSGRLDRKIEFPMPNEEARARIMQIHSRKMN	375	P17980	PSMC3
279	QLDGFQATKNIKVMATNRDILDSALLRPGRIDRKIEFPMPNEEARLDILKIHRSKMN	338	P62195	PSMC5
263	QMDGFDTLHRVKMIMATNRPDTLDPALLRPGRDLDRKIHIDLPNEQARLDILKIHAGPITK	322	P62333	PSMC6
295	QMDGFDQNVNVKIMATNRPDTLDPALLRPGRDLDRKIEFPLPDRRQKRLIFSTITSKMNL	354	P43686	PSMC4
	: . * : . : * * : * : * : * : . * .			
375	ADDVTLDDLIMAKDDLSGADIKAICTEAGLMALRERRMKVTNEDFKKSKENVLYKKQEGT	434	P62191	PSMC1
341	ERDIRFELLARLCPNSTGAEIRSVCTEAGMFAIRARRKIAATEKDFLEAVNKVIKSYAKF-	399	Q75L23	PSMC2
376	SPDVNYEELARCTDDFNQAQCKAVCVEAGMIALRRGATELTHEYMEGILEVQAKKAN-	434	P17980	PSMC3
339	TRGINLRKIAELMPGASGAEVKGVCTEAGMYALRERRRVHVTQEDFEMAVAKVMQKDSE-K	397	P62195	PSMC5
323	HGEIDYEAIVKLSDFNGADLRNVCTEAGMFAIRADHDFVQEDFMKAVRKVADSKKLES	382	P62333	PSMC6
355	SEEVLEDYVARPDKISGADINSIQESGMLAVRENRYIVLAKDFEKAYKTVIKKDEQE-	413	P43686	PSMC4
435	PEGLYL-----	440	P62191	PSMC1
400	--SATPRYMTYN	409	Q75L23	PSMC2
435	-LQYYA-----	439	P17980	PSMC3
398	NMSIKKLWK---	406	P62195	PSMC5
383	KLDYKPV-----	389	P62333	PSMC6
414	-HEFYK-----	418	P43686	PSMC4

FIGURE 9: Alignment of human PSMC subunits 1–6. Protein sequences of the six proteasome PSMC subunits were aligned using Clustal W. \*, identical residue in all six subunits; :, conserved amino acids with strongly similar properties; ., conservation between residues of weakly similar properties. Naturally occurring variants are highlighted with grey boxes. Alternatively spliced regions are underlined. Amino acid residue numbers are shown on the left and right of each sequence and the UniProt accession number and gene name of each sequence are shown to the right of each sequence.

1	-----MAAA-----AVVEFQRAQSLL--STDREAS-----IDILH--S----	30	O00231	PSMD11
1	-----	0	O00233	PSMD9
1	-----	0	Q99460	PSMD1
1	-----	0	075832	PSMD10
1	-----	0	Q9UNM6	PSMD13
1	-----MAD-----	3	000232	PSMD12
1	-----MKQEGSARRRGAD--KAK-PPPGGGGEQEP PPPAPQD-----VEMKEEAAT	43	O43242	PSMD3
1	-----MFIKGRAPRAPPR--ERRRATRGGLRQVVAPPRALGSTSRPHFRRASVCRRCRK	53	P48556	PSMD8
1	-----MA-----	2	Q16401	PSMD5
1	-----	0	P51665	PSMD7
1	-----MEEGGRDK-APVQPQQSPAAAPGGTDEKPS-----G---K	31	Q13200	PSMD2
1	-----	0	P55036	PSMD4
1	-----	0	O00487	PSMD14
1	<u>M</u> PLENLEEEGLPK- <u>N</u> PDLR----- <u>I</u> AQ----- <u>L</u> RFLLSLP----- <u>E</u>	30	Q15008	PSMD6
31	VKRDIQENDE--EAV-----QVKEQSILELGS---LL--	57	PSMD11	
1	-----	0	PSMD9	
1	-----MITSAAGIISLLDE	14	PSMD1	
1	-----	0	PSMD10	
1	-----MKDVPGFQQSQNSGPG-----	17	PSMD13	
4	--GGSERADG--RIV-----KMEVDYSATVDQ---RL--	28	PSMD12	
44	GGGTGEADGKTAAAAAEH---SQ---RELDT-VTLEDIKEHVQLEKAVSG-----	88	PSMD3	
54	SGGLLAAS-RKMAAAAVNGAAGFSSSGPAATSG-AVLQAATGMYEQLKGEWNR-----	104	PSMD8	
3	-----AQALALLREVARLE-----	16	PSMD5	
1	-----	0	PSMD7	
32	ERRDAGDKDKEQELS-----EEDKQLQDELEMLVERLGE	65	PSMD2	
1	-----	0	PSMD4	
1	-----	0	PSMD14	
31	<u>H</u> RGDAAVRDELMAAVRDNNMAPYYEALCKSLDWQIDVDLLNKMKANEDELKRLDEELED	90	PSMD6	
58	-----AKTG--QAAELGGLLK YVRP--FLNSISKAKAA	86	PSMD11	
1	-----MSDEEARQSG-	10	PSMD9	
15	DEPQLKEFALHKLNAVVDFAE-----ISESVDKIEVLYED--E-GFRSRQFAA	61	PSMD1	
1	-----	0	PSMD10	
18	-----QPAVWHRLEELYTKKL-----WHQLTL	39	PSMD13	
29	-----PECA--KLAKEGRLQEVIET--LLSLEKQTRTA	57	PSMD12	
89	-----KE-----PRFVLRALRMLPSTSRRLNH	110	PSMD31	
05	-----KSPNLKSGEELGRKLVLLELNLFLTGTGLTK-	138	PSMD8	
17	-----APLEELRA-----LHSLVQA--VPLNELRQQAA	42	PSMD5	
1	-----	0	PSMD7	
66	KDTSLYRPALEELRRQIRSTTSMTSVPKPLKFLRPHYGKLKEIYEN--MAPGENKRFAA	123	PSMD2	
1	-----	0	PSMD4	
1	-----	0	PSMD14	
91	AEKNLGESEIR-----D---AMMAKAEYLCRIGDKEGALTAFRK-----TYDK	130	PSMD6	
87	RLVRSLLDLFLDMEA-----ATGQEVE-----LCL-	111	PSMD11	
11	-----	10	PSMD9	
62	LVAS-KVFYHLGAFEESLNYALGAGDLF---NVNDNSEYVE-----TIIAK-	103	PSMD1	
1	-----	0	PSMD10	
40	<u>Q</u> V---LDFVQDPCFA---QGDGLIK---LYENFISEFEHRVNPLSLVEIILHVVRQM	87	PSMD13	
58	S---D-MVSTSR---ILVAVVK-----MCY-	75	PSMD12	
111	YV---LYKAVQGFFT---SNNATRD---FLLPF-----LEEP	138	PSMD3	
139	QQ---LILARD-----I	147	PSMD8	
43	ELRLGPLF-----	50	PSMD5	
1	-----	0	PSMD7	
124	DIIS-VLAMTMSGERECLKYRLVGSQEE---LASWGHEYVR-----HLAGE-	165	PSMD2	
1	-----	0	PSMD4	
1	-----	0	PSMD14	
131	T-----VALGHRLDIVFYLLRIGLFYMDNDLITRNTAK-----SLI---	168	PSMD6	
112	-----ECIEWAKSEKRTFLR-----	126	PSMD11	
11	-----	10	PSMD9	
104	-----CIDHYTKQCVENADLPEGEKKPIDQRLEGIVNKMFORCLDDHKYKQAIGIALE	156	PSMD1	
1	-----	0	PSMD10	
88	TDPNVALTFLEKTRKVKSS---DEAVILCK-----TAIGALKL-----N	124	PSMD13	
76	-----EAKEDLLNENIMLL-----	90	PSMD12	
139	MDTEADLQFRPRTGKAAS TPLLPEVEAYLQL-----LVVIFMM-----N	177	PSMD3	
148	LEIGA--QWS-----ILRKDIPSFER-----	166	PSMD8	
51	-----	50	PSMD5	
1	-----	0	PSMD7	
166	-----VAKEWQELDDAEKVQ-REPLTLVKEIVPYNMAHNAEHEACDLLME	210	PSMD2	

FIGURE 10: Continued.



1	-----	0	PSMD4
1	-----	0	PSMD14
169	-----EEGGDWDRRN---RLKVY-----QGLYCVAIRDFKQAAELFLD	203	PSMD6
127	-----QALEARL-----VSLYFDTKRYQEA---LHLG---SQ	152	PSMD11
11	-----	10	PSMD9
157	TRRLDVFEKTILESNDVPGM-----LAYSCLKMSLMQNKQFRNK-----VLRVLVK	203	PSMD1
1	-----	0	PSMD10
125	IGDLQVTKETIEDVEEMLNNLP-----GVT <b>SV</b> -----HSRFYDLS---SKYYQT-IG	167	PSMD13
91	-----SKRRSQLK-----QAVAKMVQQCCTYVEEITDLPIK---LR	123	PSMD12
178	SKRY---KEAQKISDDLQKISTQNRALDLVAAKCYYY-HARVYE-----FLDK-LD	225	PSMD3
167	-----YMAQLKCYFF-DYKEQLPE---SAYMHQLLG	193	PSMD8
51	-----	50	PSMD5
1	-----	0	PSMD7
211	IEQVDMLEKDIDENAY-----AKVCLYLTSCVNYVPEPENSALLRCALG	254	PSMD2
1	-----	0	PSMD4
1	-----	0	PSMD14
204	TVST--FT--SYELMDYKTF-----VTYTVYVSMIAL-----	231	PSMD6
153	LLRELK-----KMDDKA---LLVEVQ-----	170	PSMD11
11	-----	10	PSMD9
204	IYMNLEKPDFINVCQCLIFLDDPQAVSDIL-----	233	PSMD1
1	-----MEGCV-----	5	PSMD10
168	NHASYY-----KDALRFLG-CVDIKDLPVSEQQERA---FTLG---LAG	204	PSMD13
124	LIDTLR-----MVTEGK---IYVEIERARLTKTLATIKEQNGDVKEAASILQ	167	PSMD12
226	VVRSFL-----HARLRTATLRHDADG---QATLLN-----LL-----LRN	257	PSMD3
194	LNLLFL-----LSQNRVAEFHTELERLPAKDIQTNVYIKHPVS-----LEQ	234	PSMD8
51	-----S	51	PSMD5
1	-----	0	PSMD7
255	VFRKFS--RFPEALRLALMLNDMELVEDIFTSCKDVVVQKQMAFMLGRHGVFLELS---	308	PSMD2
1	-----	0	PSMD4
1	-----	0	PSMD14
232	-----E-----	232	PSMD6
171	LLESKTYHAL-----	180	PSMD11
11	-----	10	PSMD9
234	-----EKLVKEDNLLMAYQICFDLYESA-SQQFLSSVIQNLRTVGTPIASVP---	279	PSMD1
6	-----SNLMVCNLAYSGK-LEEL-----	22	PSMD10
205	<b>LLGEGVFNFGEL</b> -----LMHPVLES LRNTDRQWLI-----	234	PSMD13
168	ELQVETYGSMEKKERVEFILEQMRLCLAVKDYIR-TQIISKINTKFF-----	214	PSMD12
258	YLHYSLYDQAEK-----LVSKSVFPEQANNNEWAR	287	PSMD3
235	YLMEGSYNKV-----FLAKGNIPA-----ES-----	255	PSMD8
52	LL-----NENHREKTTLCVSILERL-- <b>L</b> QAMEPVH-----	79	PSMD5
1	-----	0	PSMD7
309	-EDVE <b>E</b> YEDLT-----EIMSNVQLNSNFLALARELDIMEPKVPDDIY-----	349	PSMD2
1	-----MVLESTMVCVDNSEYMRNGDFLPTRLQAQQDAVNIVCHSKTRS	43	PSMD4
1	-----	0	PSMD14
233	-----	232	PSMD6
181	-----	180	PSMD11
11	-----	10	PSMD9
280	-GSTNTGTVPGSEKSDSMETEEKTSSAFVGKTPEASPE---PKDQTLKMIKILSGEMA	334	PSMD1
23	-----	22	PSMD10
235	-DTL-----	237	PSMD13
215	-QE-----ENTEKLKLKYNL-----	229	PSMD12
288	-YLYYTGRIKAIQLEYSEAR--RT-MTNALRKAPQHTAVGFKQTVHKLLIVVELLLGEIP	343	PSMD3
256	-YTFEIDIL-----LDTIR--DE-IAGCIEKA-----YEK-----ILFTEAT	288	PSMD8
80	-----	79	PSMD5
1	-----	0	PSMD7
350	-KT-----HLE---NNRFGGS-----	361	PSMD2
44	NPENNVGLIT-LANDCEVLT-----TLTPD-----TGRIL	72	PSMD4
1	-----	0	PSMD14
233	-----	232	PSMD6
181	-----SNLPKARAAL TSA--RTTANAIYCPPKLQATL	210	PSMD11
11	-----G	11	PSMD9
335	IELHLQFLIRNNNTDLMILKNTKDAVRNSVCHTATVIANSEFMHCGTTSDF-----	385	PSMD1
23	-----KES-----ILADK--SLATRTDQD-----	39	PSMD10
238	-----	237	PSMD13

FIGURE 10: Continued.

230	-----MIQLDQHEGSYLSI--CKHYRAIYDTPCIQ---	257	PSMD12
344	DR-----LQFRQPSLKRSL	357	PSMD3
289	RI-----LFFNTPKKMT---	300	PSMD8
80	-----VA-----RNLRV	86	PSMD5
1	-----	0	PSMD7
362	-----GSQVDSARMNLASSFVNGFVNAAFQGDK-----	389	PSMD2
73	SKLH-----TVQPKGKITFCTGIRVA-----	93	PSMD4
1	-----	0	PSMD14
233	-----	232	PSMD6
211	DMQSGIIHAAEEKDWKTAYS-----FYEAFFGYDSIDSP-----KAITS	250	PSMD11
12	SSQAGV-----VTVS-----DVQELMRKE-----	31	PSMD9
386	-----LRDNLEWLARATNWAKFTATASLGVHKGHEKEALQLMATYLPKDTSP----	433	PSMD1
40	-----SRTALHWACSAGHTEIVEFLLQLGVFVNDKDD-----	71	PSMD10
238	-----YAFNSGNVERFQT-----	250	PSMD13
258	-----AESEKWQQALKS-----V-----	270	PSMD12
358	-----MPYFLLTQAVRTGNLAKFNQ-----	377	PSMD3
301	-----DY-----AKKRG-----	307	PSMD8
87	DLQRGLIHPDDSVKILTLS-----QIGRIVENS-----	115	PSMD5
1	-----	0	PSMD7
390	-----LLTDDGNKWLYKNKDHGMLSAAASLGMILLWDVDGGLTQIDKYLYSS--EDYIK	441	PSMD2
94	-----HLALKHRQGNHMKRIIAFVGSPVEDNEKDVLKAKRLKKEKVNVDIIN	142	PSMD4
1	-----	0	PSMD14
233	-----	232	PSMD6
251	L-----KYMLLCKIMLN-----TPEDVQALV-----SG-----	273	PSMD11
32	-----EI-----	33	PSMD9
434	-G-----SAY-----QEGGGLYALGLIHANH--GGDIIDYLLNQLKNASNDIVRHGG-	477	PSMD1
72	-----AGWSPLHIAASAG--RDEIVKALLGKG--AQVNAVQNQG-	106	PSMD10
251	-----LKTAWG-----QQ--PDLAANEA---QLLRKIQL---LC	276	PSMD13
271	V-----LYVILAP-FDN---EQSDLVHRI-----SG-----	292	PSMD12
378	-----VLDQFG-----EKFQADGTYTLI---IRLRHNVIKTGVR	408	PSMD3
308	-----WVLGPNNYYSFA---S-----	320	PSMD8
116	-----AVTEILNNAELLKQIVYCIGGE	137	PSMD5
1	-----	0	PSMD7
442	S-----GALLACGIVNSGVRNECDPALALLSDYVLHNSNTMRLG-S	481	PSMD2
143	FGEEEVNTEKLTAFVNTLNGKDGTSGLVTVPP--GPSLADALIS---SPILAGEGGA	195	PSMD4
1	-----MDRLLRLGGG	10	PSMD14
233	-----	232	PSMD6
274	--KLAL-----RYA---GRQTEALK-----CVAQAS	294	PSMD11
34	-----EAQ-----IKANYDVLES-----	46	PSMD9
478	SLGLGL-----AAM---GTARQDVYDLLKTNLYQDD	505	PSMD1
107	CTPLHY-----AAS---KN-RHEIAVM-----	124	PSMD10
277	LMEMTFTRPANHRQLT-----FEEIAKSAK-ITV	304	PSMD13
293	--DKKL-----EEI---PKYKDLLK-----LF	309	PSMD12
409	MISLSYS-----RIS-----LADIAQKLQLDSP	431	PSMD3
321	-----QQQKP	325	PSMD8
138	NLSVAKAAIKSLSRISLTQAGLEALFESNLLDDLKSMVMTNDIVRYRYVELIEISSVSP	197	PSMD5
1	-----M-----PEL---AVQKVVV-----	11	PSMD7
482	IFGLGL-----AYA---GSNREDVLTLLLPVMGDSK	509	PSMD2
196	MLGLGASD-----FEFGVDPSAD--PEL---ALALRVSMEE--EQRQRQEE	233	PSMD4
11	MPGLGQGP-----P-----TDA---PAV---DTAEQVYIS-----	34	PSMD14
233	-----	232	PSMD6
295	KNRSLADFEKALTDY-----RAELRDDPIISTH-----	322	PSMD11
47	-----	46	PSMD9
506	AVT--GEAAGLALGLV-----MLGSKNAQAI-----EDMVGYAQETQH	541	PSMD1
125	-----L-----LEGGANPDAK-----D---HYEATAMH	144	PSMD10
305	NEVELLMKALSVGLV-----KGSIDEVD-----KRVHMTWV	336	PSMD13
310	TMELMRWSTLVEDY-----GMELRKGSLESPE-----TDVFGSTEEG	347	PSMD12
432	EDAEFIVAKAIRDGI-----EASINHEK-----GYVQS---	460	PSMD3
326	EDTTIP-STELAKQVI-----EYARQ---	345	PSMD8
198	ESLNYCTTSGVLQTLRELGTEDVLVRATCIEMVTSLA---YTHHGRQYLA-----	245	PSMD5
12	-----HPLV-----LLSVVD-----HFNR-----	25	PSMD7
510	SSMEVAGVTALACGMI-----AVGSCNGDVTSTI-----LQ--TIMEKS	546	PSMD2
234	EARRAAAASAAEAGIA-----TTGTEDSDDALKMTISQQEFGRGTGLPDLSSMTEE	284	PSMD4
35	-----SLALLKML	42	PSMD14
233	-----	232	PSMD6

FIGURE 10: Continued.

323	---LAKLYDNLLEQ-----NLRVIEPFS-----	343	PSMD11
47	-----QKGIGMNEPLV--DCEGYPR	64	PSMD9
542	E----KILRGLAVGIALVYGRME-----EAD-ALIESLCRDKDP---I	577	PSMD1
145	R----AAAKGNLKMIIHILYYKAST-----NIQDTEGN-TPLHLACDEERVVEAKL	190	PSMD10
337	QPRVLDLQO-----I---K-----	347	PSMD13
348	EKRWKDLKNRVVEH-----NIRIMAKYYT-----	371	PSMD12
461	-KEMIDIYS-----TREPQL-----	474	PSMD3
346	-LEMIV-----	350	PSMD8
246	-----QEGVIDQISNIIVGADSDPFSSFYLPGFVKFFGNLAVMDSPQQ--ICERYPI	295	PSMD5
26	-----IGKVGNGKRVVGVLLGSWQK-----KVLDSVNS-FAV--PFDEDDKDDSVW	68	PSMD7
547	ETELKDTYARWLPLGLGLNHLGKGEA-----I-EAILAALEVSEPF-----	588	PSMD2
285	E----QIAYAMQMSLQGAEFQGAES-----ADIDASSAMD-TSEPAKE--EDDYDV	328	PSMD4
43	K-----HGRAGVPMEVMGLMLGEFVD-----DYTVRVIDVFAM--P--QSGTGVSVSE	85	PSMD14
233	-----	232	PSMD6
344	-----RV-----QIEHISSLIKLSKADVERKLSQ-----	367	PSMD11
65	SDVDLYQVPTARHN-IICLQN-----DHKAVMKQVEEALHQLHAR-DKEKQ-----	108	PSMD9
578	-----LRRSGMYTVAMAYCGSGNKAIRRLHVAVSDVNDDVRR-----	616	PSMD1
191	-----LVSQGA-----SIYIENK-----EEK-----TPLQ-----	210	PSMD10
348	-----GMKDRLEFWCTDV-----	360	PSMD13
372	-----RI-----TMKRMAQLLDLSVDESEAFLSN-----	395	PSMD12
475	-----AFHQR-ISFCLDI-----	486	PSMD3
351	-----	350	PSMD8
296	FVEKVFEMIESQDPTMIGVAV-----DTVGILGSNVE-----GKQ-----	330	PSMD5
69	FLDHDYLENMYGMFKKVN-----ARERIVGWYHTGPKLHKND-----	105	PSMD7
589	-----SFANTLVDVCAYAGSGNVLKVQQLLHICSEHFDSKEKEEDKDKKEKKDKDKK	640	PSMD2
329	MQDPEFLQSVLE-----	340	PSMD4
86	AVDPVFQAKML-----DMLKQTGRPEMVVGWYHSHPGFGCWL-----	122	PSMD14
233	-----	232	PSMD6
368	-----MILDKKFHG-----ILDQGEGVLIIFDEPPVDKTYEAALETI---	404	PSMD11
109	-ARDMAE-----AH-----KEAMSRKLGQSESQGPFR-----AFAKVNSISPG	145	PSMD9
617	-----AAVESLGFILFR-----TPEQCPSVSVL-----	639	PSMD1
211	-----VAKGGLGLILKR-----MVEG-----	226	PSMD10
361	-----	360	PSMD13
396	-----LVVNKTIFA-----KVDRLAGIIN-FQRPKDPNNL-----L---	425	PSMD12
487	-----	486	PSMD3
351	-----	350	PSMD8
331	-VLQKTG-----TR-----FERLLMRIGHQSKNAPVELKIRCLDAISSLLYL	371	PSMD5
106	-----IA-----INEL-----MKRYCPNSVLV-----	122	PSMD7
641	EAPADMGAHQGVAVLGIALIAMGEEIGAEMALRTFGHLLRYGEPTLRRRAVPLALALISVS	700	PSMD2
341	-----	340	PSMD4
123	-----	122	PSMD14
233	-----RPDLREKVIKGAET-----	246	PSMD6
405	-----QNMSKVVDs-----LY--NKAKKL-----	421	PSMD11
146	SPAS-----	149	PSMD9
640	-----LSESYNPHVRYGAAMALG-----ICCACT-----GNKE--AI	669	PSMD1
227	-----	226	PSMD10
361	-----	360	PSMD13
426	-----NDWSQKLNSLM-----SLV--NKTTHL-----	445	PSMD12
487	-----	486	PSMD3
351	-----	350	PSMD8
372	PPEQQTDD-----	379	PSMD5
123	-----IIDVKPKDLGLPTEAYISVEEVHDDGTPSTKTFEHTVSEIGAEAEAEV	170	PSMD7
701	NPRLNILDTLKFSHDADPEVSYNSIFAMGMV---GSGTNN-----	738	PSMD2
341	-----	340	PSMD4
123	-----SGVDI-----NTQQSFEALS-----ER	139	PSMD14
247	-----	246	PSMD6
422	-----T-----	422	PSMD11
150	-----IAGLQVDDEIV-----	160	PSMD9
670	---NLLEPMTNDPVNYVRQGALIASALIMIQQTEITCPKVNQFRQLYSKVINDKHDDVMA	726	PSMD1
227	-----	226	PSMD10
361	-----	360	PSMD13
446	-----IA-----KEEMIHNLQ-----	456	PSMD12
487	-----	486	PSMD3
351	-----	350	PSMD8
380	-----LLRMTESWFSLSRDPLE-----	397	PSMD5

FIGURE 10: Continued.

171	GVEHLLRDIKDTTVGTLSQ-----RITNQVHGLKGLNSKLLDIR-----	209	PSMD7
739	-----AR-----LAAMLRQLAQYHA-----KDPNN	758	PSMD2
341	-----	340	PSMD4
140	AVAVVVD-----PIQSVKKG-VV-----IDAFRLINANMMVLG-----	171	PSMD14
247	-----LEVLSLPAV-----	256	PSMD6
423	-----	422	PSMD11
61	EFGSVNTQNFQSLHNI--GSVVQHSEGEKPLNVTVIRRGEEKHQLRLV----PTRWA--GK	211	PSMD9
727	KFGAILAQGILDAGGHNVITISLQ-S-----RTGHTHMPSVVGVLVFTQFWF----	771	PSMD1
227	-----	226	PSMD10
361	-----	360	PSMD13
457	-----	456	PSMD12
487	-----	486	PSMD3
351	-----	350	PSMD8
398	LFRGISSQPFPELHCA--ALKV-----FTATA----NQPWA--QK	429	PSMD5
210	SYLEKVATGKLPIN-HQIIYQL-----QDVFNLLPDV-SLQEFVKAF-----	249	PSMD7
759	LFMVRLAQGLTHLGKGTLTLCPYHS-----DRQLMSQVAVAGLLTVLVSFLDVRN	808	PSMD2
341	-----	340	PSMD4
172	-----HEP-RQTT-S-----NLGHLNKPSIQALIHGLNRHY-----	200	PSMD14
257	-----RQYLFSLYEC-----RYSVFFQS---L---AVVEQEMKK	284	PSMD6
423	-----	422	PSMD11
212	GLLGCNII--PLQR-----	223	PSMD9
772	-WFPLSHFLS---LA---Y-----TPTCVIGLNKDLK--MPKVQYKSN--CKPSTFA	812	PSMD1
227	-----	226	PSMD10
361	-----KSMEMLVHEQ-----AHDIL-----	375	PSMD13
457	-----	456	PSMD12
487	-----HNMSVKAMRFPPKSYNKDLE-----	506	PSMD3
351	-----	350	PSMD8
430	LMFNSPGFVEYVVDRSV-----EHDKASKDAKYELVKALANSKTIAEIFG	474	PSMD5
250	-YLKTNQMV---VV---Y-----LASL-----IRSVVA	271	PSMD7
809	IILGKSHYVLYGLVAA---M-----QPRMLVTFDEELR-----PL---	840	PSMD2
341	-----NLPG-----	344	PSMD4
201	-YSITINYRK---NE---L-----EQKMLNLHKKSWMEGLTLQDYSEH--CKHNESV	244	PSMD14
285	DWLFAPHYRYVREMRIHAYSQQLLESYRSLTLGYMAEAFGVG-----	326	PSMD6
423	-----	422	PSMD11
224	-----	223	PSMD9
813	<u>YPAPLEVP-KEKEKEKVSTA---VLSITAKAKKKEKEKEKEEKEVDEAEKKEEKEKK</u>	868	PSMD1
227	-----	226	PSMD10
376	-----T-----	376	PSMD13
457	-----	456	PSMD12
507	-----SAEERREREQQDLEFAKEMA--EDD	529	PSMD3
351	-----	350	PSMD8
475	NPN-----	477	PSMD5
272	LHNLINNKIANRDAEKKEGQ---EKEESKKDRKEDKEK-DKDKEKSDVKKEEKKE---KK	324	PSMD7
841	---PVSVR--VGQAVDVVGQAGKPK-----	860	PSMD2
345	---VDPNNEAIRNAMGSLASQATKDGKKDKKEEDKK-----	377	PSMD4
245	VKEMLE-----LAKNYNKAVEEEDKMTPEQLAIKNVGKQDPKRH--	283	PSMD14
327	-----VEFIDQELSRF-IAAGRLHC-----KIDKVNEIVETNRPDSKNWQYQE-----	368	PSMD6
423	-----	422	PSMD11
224	-----	223	PSMD9
869	KEPEPNFQLLDNPARVMPAQLKVLTPETCRYQPFKPLSIGGIIILKDTSEDIIEELVEPV	928	PSMD1
227	-----	226	PSMD10
377	-----	376	PSMD13
457	-----	456	PSMD12
530	DDSF-----	534	PSMD3
351	-----	350	PSMD8
478	-----YLRLRTYLSEGPYYVKPVS-----TT	498	PSMD5
325	-----	324	PSMD7
861	--TITGFQTHTPVLLAHGER---AELATEEFLPVTPILEGFVILRKNPNYDL-----	908	PSMD2
378	-----	377	PSMD4
284	LEEHDVDMTNSNIVQCLAAMLN-----TVVEK-----	310	PSMD14
369	--TIKKGDLLLNRVQKLSRVI---NM-----	389	PSMD6
423	-----	422	PSMD11
224	-----	223	PSMD9
929	AAHGPKIEEEEQEPEPPEFFFYIDD	953	PSMD1
227	-----	226	PSMD10
377	-----	376	PSMD13
457	-----	456	PSMD12
535	-----	534	PSMD3
351	-----	350	PSMD8
499	AVEGAE-----	504	PSMD5
325	-----	324	PSMD7
909	-----	908	PSMD2
378	-----	377	PSMD4
311	-----	310	PSMD14
390	-----	389	PSMD6

FIGURE 10: Alignment of human PSMD subunits 1-14. Protein sequences of the thirteen proteasome PSMD subunits were aligned using Clustal W. No residues are conserved in all PSMD subunits. Naturally occurring variants are highlighted with grey boxes. Alternatively spliced regions are underlined. Amino acid residue numbers are shown on the left and right of each sequence and the UniProt accession number and gene name of each sequence are shown to the right of each sequence.



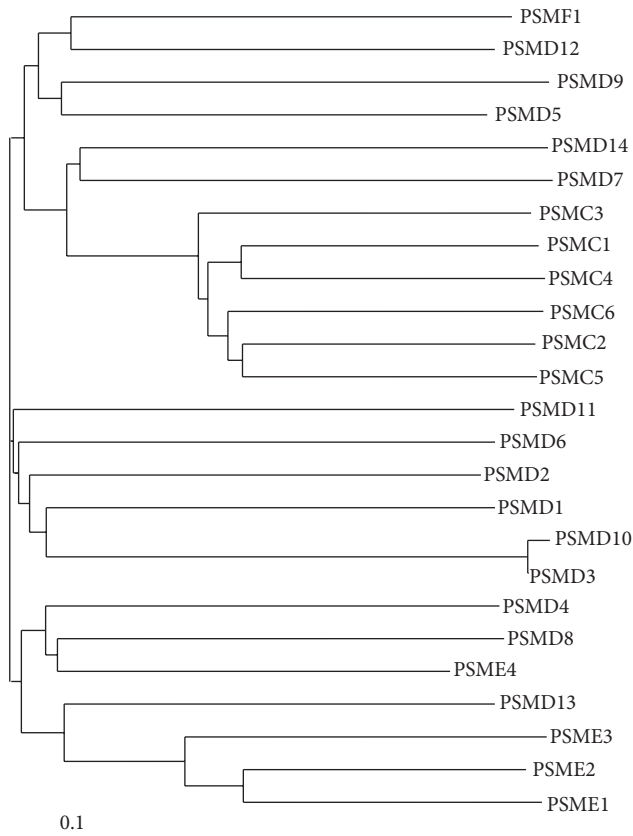


FIGURE 11: Phylogenetic tree of human PSMC, PSMD, PSME, and PSMF proteasome subunits. Phylogenetic tree was generated using Clustal W2 phylogeny [88] and image obtained using TreeView [89]. The UniProt accession numbers used for the alignment of proteasome subunits are given in Figures 9, 10, 12, and 13.

**3.3. *PSMD3*.** The *PSMD3* gene encodes a member of the proteasome 19S regulatory cap, Rpn3. Rpn3 is one of the non-ATPase subunits and is composed of 534 amino acids. *PSMD3* variants are associated with insulin resistance in different populations and these relationships are likely to be modified by dietary factors [76]. Insulin resistance is critical in the pathogenesis of chronic diseases such as CAD, hypertension, inflammation, and T2DM [125, 126].

**3.3.1. Diabetes as Related to Insulin and Dietary Intake.** The UPS has been shown to regulate insulin signal transduction via several mechanisms, including regulation of glucose transporters, ubiquitination of the insulin receptor, and degradation of insulin receptor substrate [127]. Ten SNPs covering 90% the genetic variations in or near *PSMD3* were investigated. Using two independent groups: the GOLDN (Genetics of Lipid Lowering Drugs and Diet Network) study which included 820 participants of Northern European origin, and the BPRHS (Boston Puerto Rican Health Study) study, which included 844 participants recruited by the Boston Puerto Rican Center for Population Health and Health Disparities, the minor C allele carriers of the SNP rs4065321 had a higher homeostasis model assessment of insulin resistance than noncarriers in males of both studies.

An interaction between SNP rs709592 and dietary carbohydrate on a higher homeostasis model assessment of insulin resistance subjects with the T allele was detected in the GOLDN group. SNPs rs4065321 and rs709592 both significantly interacted with dietary factors in the GOLDN study.

**3.3.2. White Blood Cell Count.** Total white blood cell (WBC) and neutrophil counts vary among different ancestry groups and are lower among individuals of African descent [128]. Measuring WBCs in humans is universally used in diseased and asymptomatic patients to identify or predict chronic disease. WBCs are made up mostly of neutrophils, which are a key component of the innate immune system as an early line of defense against invading microorganisms. Very low numbers of neutrophils have been shown to make patients susceptible to bacterial infections and can lead to lethal conditions [129]. *PSMD3* has also been reported to be associated with white blood cell counts [130–132]. The rs4065321 of *PSMD3-CSF3* region was associated with WBC count in African American and other populations. GWA analysis of 13,923 subjects in the electronic Medical Records and Genomics (eMERGE) Network identified two regions each unique to subjects of genetically determined ancestry to the African continent or to the European continent [131]. One of these regions contained the *PSMD3* intronic SNP rs4065321 (in persons of European ancestry) that was found to be significantly associated with WBC count [131]. A GWA study in 5771 Japanese and a replication study using independent 1894 Japanese identified rs4794822 in *PSMD3-CSF3* as being significantly associated with neutrophil count [132]. The SNP rs4794822 in *PSMD3-CSF3* was not associated with lymphocyte, monocyte, eosinophil, or basophil counts, suggesting a specific association with neutrophils [132].

**3.4. *PSMD7*.** *PSMD7*, the 19S proteasome non-ATPase regulatory subunit 7, encodes the protein Rpn8, which is involved in the ATP-dependent degradation of ubiquitinated proteins [133]. Rpn8 is a 324 residue protein which is modified by acetylation of K204 and K214 and may be involved in viral replication [84, 134]. The HIV-1 accessory gene product Vpr interacts with MOV34 (homologous to *PSMD7*) [134]. The induction of cell cycle arrest at the G2/M phase border by Vpr correlated with a change in the subcellular localization of MOV34 from a nuclear to a perinuclear localization as well as the inhibition of the maturation promoting factor-associated histone H1 kinase activity. These results suggest that *PSMD7* may be involved in the regulation of the cell cycle and is a likely cofactor for HIV-1 Vpr [134].

**3.4.1. Ankylosing Spondylitis.** Blood samples from 185 Chinese patients with AS (149 male) and 516 healthy controls (412 male) showed that SNP rs17336700 of *PSMD7* is significantly associated with AS in a Chinese population [18]. Two mutations, 392-187C → T and 392-192delTC, were detected once in the AS patients. The SNP rs17336700 had a minor allele frequency of 13.0% and was significantly increased in AS patients relative to controls. Allele-wise analysis also indicated a higher frequency of the rs17336700 C allele in

patients when compared to controls [18]. Human liver biopsy samples from 73 patients (containing eight rs17336700 TC heterozygotes) showed that *PSMD7* gene expression in the TC group was 1.88-fold higher than that of the TT group.

**3.5. *PSMD13*.** *PSMD13* is one of the least understood proteasome genes. It codes for a 376 amino acid protein called Rpn9 which is part of the 19S regulatory cap that is involved in the ATP-dependent degradation of ubiquitinated proteins [135]. Two isoforms of *PSMD13* are produced in humans by alternative splicing and its translated product Rpn11 is acetylated at K298 [84].

**3.5.1. Platelet Traits.** Platelet traits have been shown to be highly heritable and well established as being important for the pathogenesis of atherothrombosis and cancer. Investigation of genetic variants associated with platelet traits identified five chromosomal regions associated with variation in the number of circulating platelets (PLT) and eight associated with mean platelet volume (MPV) variation with genome-wide significance [136]. Several SNPs near the telomere region of chromosome 11p were associated with PLT. This region contains six genes, including *PSMD13*. Like most complex diseases multiple genetic loci influence interindividual variation in platelet traits.

**3.6. *PSMD14*.** *PSMD14* codes for Rpn11 which is a metallo-protease (binds zinc) that specifically cleaves K63-linked but not K48-linked polyubiquitin chains [137]. As part of the 19S, Rpn11 is involved in the ATP-dependent degradation of ubiquitinated proteins. Rpn11 is a 310 residue protein which is important for recycling Ub from proteasome substrates and is also a key deubiquitinating enzyme for regulating Ub conjugates generated in response to DNA damage as well as several aspects of the mammalian DNA double-strand break response [138]. In *Schizosaccharomyces pombe*, the yeast equivalent of *PSMD14* (POH1), has been shown to confer pleiotropic drug resistance to taxol, doxorubicin, 7-hydroxystaurosporine, and ultraviolet light when transiently overexpressed in mammalian cells [139]. These experimental data all suggest that Rpn11 is important in cellular susceptibility to cytotoxic agents. Rpn11 is known to be phosphorylated on S150 and S224 [140].

**3.6.1. Intellectual Disability.** *PSMD14* is part of a 0.4 Mb region of 2q24.2 that is associated with intellectual disability and short stature [141]. An 18-year-old male with mild intellectual disability and short stature had a 0.422 Mb deletion on 2q24.2 which was detected by comparative genomic hybridization. This deleted region included three genes: *TBR1*, *TANK*, and *PSMD14* [141]. While it is not known if all three genes are important for the phenotype, the association of other proteasome genes with intellectual disability suggests that *PSMD14* is a possible candidate gene that may be associated with intellectual disability. The proteasome is likely involved in intellectual disabilities indirectly by altering the degradation of key signaling proteins important for intellect.

## 4. Polymorphism Associated with Reduced Risk of Disease

**4.1. Multiple Sclerosis.** Multiple sclerosis (MS) is a common but complex autoimmune disease which displays accumulated immunoproteasomes in plaques of affected brain areas. The immunoproteasome *PSMB9* codon 60HH variant was observed to have a reduced risk of developing MS in HLA-A\*02+ Italian females [142]. Although the role of the proteasome in autoimmune diseases is only partly understood, the treatment of autoimmune diseases with proteasome inhibitors has been successful in animal models [143, 144]. Production of MHC class I-restricted epitopes by the proteasome is a key step in the activation and regulation of autoreactive CD8+ T cells. Immunoproteasomes carrying the *PSMB9* 60H allele show a lower amount of the HLA-A\*0201 restricted epitope myelin basic protein residues 111–119 (MBP<sub>111–119</sub>) *in vitro* [142]. It is possible that the altered proteasome-dependent production of a specific MBP epitope presented on the MHC class I may be important in MS pathogenesis [142].

**4.2. Effect of Reduced Copy Number of a Proteasome Gene on Disease Susceptibility.** Another way by which the proteasome may contribute to disease is by increasing disease related liability in cells, thereby resulting in reduced numbers of diseased cells. A distinct class of cancer-specific liabilities resulting from genome instability was recently reported [145]. Utilization of both genome wide copy number and loss of function data (RNAi profiles) from 86 cancer cell lines identified predominantly proteasome, spliceosome, and ribosome components that were associated with associated with copy-number loss [145]. Cells containing partial *PSMC2* copy-number loss lack a proteasome complex composed of the protein product of *PSMC2*, Rpt1, and three other 19S subunits and eventually die after *PSMC2* suppression [145].

## 5. Polymorphisms in Genes That Code for Proteins Which Directly Interact and Affect Proteasome Function

Besides directly having polymorphisms on proteasome subunits which affect proteasome function, proteasome interacting proteins are also likely to have mutations that affect proteasome function. An E201 deletion in the proteasome 26S ATPase subunit 3-interacting protein (PSMC3IP), which is highly expressed in testis and colon, has been associated with XX ovarian dysgenesis [146]. XX ovarian dysgenesis is characterized by primary amenorrhea, lack of spontaneous pubertal development, hypergonadotropic hypogonadism, and uterine hypoplasia as a result of streak gonads [146]. PSMC3IP enhances the meiotic recombination protein DMCI-mediated strand exchange needed for pairing homologous chromosomes during meiosis and has been shown to modulate the activity of proteasomes through association with PSMC3 [146–148]. However, the effect of the E201 deletion on proteasome function has not been determined.

Polymorphisms in other proteins such as the proteasome maturation protein (POMP) are also known to be associated with rare diseases. A one base pair deletion (–95C) in POMP



10	20	30	40	50	60	
MEPAERAGVG	EPPEPGRPE	PGPRGFVPQK	EIVYNKLLPY	AERLDAESDL	QLAQIKCNLG	60
RAVQLQELWP	GGLFWTRKLS	TYIRLYGRKF	SKEDHVLFIK	LLYELVSIPK	LEISMMQGF	120
RLILNLLKKK	ELLSRADLEL	PWRPLYDMVE	RILYSKTEHL	GLNWFNPNSVE	NILKTLVKSC	180
RPYFPADATA	EMLEEWRLPM	CPFDVTMQKA	ITYFEIFLPT	SLPPELHHKG	FKLWFDELIG	240
LWVSVQNLPO	WEGQLVNLFA	RLATDNIGYI	DWDPYVPKIF	TRILRSLNLP	VGSSQVLVPR	300
FLTNAVDIGH	AVIWITAMMG	GPSKLVQKHL	AGLFNSITSF	YHPSNNGRWL	NKLMKLLQRL	360
PNSVVRRLHR	ERYKKPSWLT	PVPDSHKLTD	QDVTDFVQCI	IQPVLLAMFS	KTGSLEAAQA	420
LQNLALMRPE	LVIPPVLERT	YPALETLTPE	HQLTATLSCV	IGVARSLVSG	GRWFPEGPTH	480
MLPLLMRALP	GVDPNDFSKC	MITFQFIATF	STLVPLVDCS	SVLQERNDLT	EVERELCSAT	540
AEFEDFVLQF	MDRCFGLIES	STLEQTREET	ETEKMTHTLES	LVELGLSSTF	STILTQCSCE	600
IFMVALQKVF	NFSTSHIFET	RVAGRMVADM	CRAAVKCCPE	ESLKLFLVPHC	CSVITQLTMN	660
DDVLNDEELD	KELLWNLQLL	SEITRVDGRK	LLLYREQLVK	ILQRTLHLTC	KQGYTLSCNL	720
LHLLLRSTTL	IYPTCYCSVP	GGFDKPPSEY	FPIKDWGKPG	DLWNLGIQWH	VPSSEEVSFA	780
FYLLDSFLQP	ELVKLQHCGR	GKLEMSRDDI	LQSLTIVHNC	LIGSGNLLPP	LKGEPTNLV	840
PSMVSLEETK	LYTGLELDL	RENHREVIAT	VIRKLLNHIL	DNSEDDTKSL	FLIIKIIGDL	900
LQFQGSHEK	FDSRWKSFNL	VKKSMENRLH	GKKQHIRALL	IDRVMLQHEL	RTLTVEGCEY	960
KKIHQDMIRD	LLRLSTSSYS	QVRNKAQOTF	FAALGAYNFC	CRDIIPLVLE	FLRPDRQGV	1020
QQQFKGALYC	LLGNHSGVCL	ANLHDWDCIV	QTWPAIVSSG	LSQAMSLEKP	SIVRLFDDLA	1080
EKIHRQYETI	GLDFTIPKSC	VEIAELLQQS	KNPSINQILL	SPEKIKEGIK	RQQEKADAL	1140
RNYENLVDTL	LDGVEQRNLP	WKFEHIGIGL	LSLLLRDDR	LPLRAIRFFV	ENLNHDAIVV	1200
RKMAISAVAG	ILKQLKRTHK	KLTINPCEIS	GCPKPTQIIA	GDRPDNHWLH	YDSKTIPTRK	1260
KEWESSCFVE	KTHWGYTWP	KNMVVYAGVE	EQPKLGRSRE	DMTEAEQIIF	DHFSDPKFVE	1320
QLITFLSLED	RKGDKFNP	RFCLFKGIFR	NFDDAFLPVL	KPHLEHLVAD	SHESTQRCVA	1380
EIIAGLIRGS	KHWTFEKVEK	LWELLCPLLR	TALSNITVET	YNDWGACIAT	SCESRDPRKL	1440
HWLFELLES	PLSGEGGSFV	DACRLYVLQ	GLAQQEWV	ELLHRLKYL	EPKLTQVYKN	1500
VREIRIGSVLT	YIFMIDVSLP	NTPTTISPHV	PEFTARILEK	LKPLMDVDEE	IQNHVMEENG	1560
IGEDERTQG	IKLLKTILKW	LMASAGRSFS	TAVTEQLQLL	PLFFKIAPVE	NDNSYDELKR	1620
DAKLCLSLMS	QGLLYPHQVP	LVLQVLKQTA	RSSSWHARYT	VLTYLQTMVF	YNLFIFLNNE	1680
DAVKDIRWL	ISLLEDEQLE	VREMAATTLS	GLLQCNFLTM	DSPMQIHFEQ	LCKTKLPKKR	1740
KRDPSGVGDT	IPSAELVKRH	AGVLGLGACV	LSSPYDVPTW	MPQLLMNLSA	HLNDPQPIEM	1800
TVKKTLSNFR	RTHHDNWQEH	KQQFTDDQLL	VLTDLLVSPC	YYA	1840	

(a)

10	20	30	40	50	60	
MAGLEVLFAS	AAPAITCRQD	ALVCFLHWEV	VTHGYFGLGV	GDQPGPNDDK	SELLPAGWNN	60
NKDLYVLRYE	YKDGSRKLLV	KAITVESSMI	LVNLEYGSQQ	VADLTNLDD	YIDAEHLGDF	120
HRTYKNSEEL	RSRIVSGIIT	PIHEQWEKAN	VSSPHREFPP	ATAREVDPLR	IPPHHPTSR	180
QPPWCDPLGP	FVVGGEDLDP	FGPRRGGMIV	DPLRSGFPRA	LIDPSSGLPN	RLPPGAVPPG	240
ARFDPFGPIG	TSPPGPNPDH	LPPPGYDDMY	L	270		

(b)

FIGURE 13: Sequences of human PSME4 and human PSMF1 subunits. Naturally occurring variants are highlighted with grey boxes. Alternatively spliced regions are underlined. Amino acid residue numbers are shown on the right of each sequence. UniProt accession numbers for PSME4 and PSMF1 are Q14997 and Q92530, respectively.

factors. People with different genetic variations sometimes respond differently to the same environmental exposure. A recent study using pooled data from 24 studies of the Breast Cancer Association Consortium (34,793 invasive breast cancers and 41,099 controls) showed that the risk of breast cancer associated with some common genetic variants varies with environmental risk factors (such as alcohol consumption and parity) [159].

## 8. Conclusions

In the last five years, genetic studies have significantly increased our basic understanding of genes associated with

diseases. Several disease-associated and promising disease-related candidate genes have been determined for diseases ranging from cardiovascular diseases to immune diseases. The known number of polymorphisms associated with disease and the number of diseases associated with polymorphisms are both likely to rise significantly over the next decade. Numerous mutations and polymorphisms in other proteasome genes (Table 1 and Figures 4, 5, 9, 10, 12, and 13) are already known, but the functional consequences of these genetic variations are not known. Several mutations in proteasome genes not associated with disease have been found in diseased tissues, such as a somatic mutation in Rpt6 (PSMC5), R60Q, found in a colorectal cancer sample [39].



Understanding whether or not these proteasome mutations are important in disease development will require basic and advanced research to determine how these mutations affect proteasome function and how they affect the cell's physiology. Another question that still needs to be answered is what factors contribute to some polymorphisms having a strong association with diseases in one or a few ethnic groups but not in other ethnic groups.

Studies involving tissues from patients have also increased our understanding of the pathophysiology of these diseases. While measurement of proteasome activity in diseased tissues is important, measurement of purified proteasome activities in these tissues is also needed to determine if the effects of the polymorphism are directly due to modulation of the proteasome or due to indirect effects. It is possible that an amino acid change in a proteasome subunit may cause altered proteasome activity by affecting its interaction with certain enzymes (e.g., preventing or reducing phosphorylation at certain sites), or by affecting weak associating proteins which alter proteasome activity. Another factor that is not yet considered when determining the role of polymorphisms on proteasome function is the large number of posttranslational modifications that occur on proteasome subunits [27, 160–162]. The heterogeneity of posttranslational modifications on proteasome subunits depends on many factors which will vary significantly between individuals. The most common posttranslational modification is possibly phosphorylation, which can be removed by nonspecific phosphatases, allowing dephosphorylated, purified proteasomes from normal and diseased tissues to be compared. Ideally, expression of wild-type and mutant proteasome subunits which are integrated into the intact 26S proteasome in a cell culture system would allow the researchers to determine if posttranslational modifications are major considerations when defining the role of SNPs in proteasome functions.

Positive associations between a polymorphism and a disease in case control association studies are often not replicated in independent studies, as the design of many studies lack the statistical power to properly detect any potential association [163]. In general, larger population sizes are needed for association studies. When large population studies are unavailable, but enough “smaller” studies are available, meta-analysis of GWA studies should be carried out. Better collaboration between research groups and even countries is needed to allow significantly larger population studies to be conducted. These larger studies are critical to help unravel the effects of environmental factors on disease related polymorphisms. Besides limited sample size, problems due to false-positive results and publication bias are still a significant problem [164].

The current standard of using phenotypic biomarkers for clinical prognosis will continue for the foreseeable future since these biomarkers integrate both genetic and nongenetic factors. Nevertheless, in the near future it is likely that genotyping for specific SNPs will be useful in clinical diagnosis and prognostic assessment of patients. SNP markers are already being used in the diagnosis of a few diseases such as Wilson disease [165]. An understanding of how the gene

polymorphisms affect proteins associated with disease will likely lead to new drug targets and therapeutic approaches.

## Conflict of Interests

The author declares that there is no conflict of interests.

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